Devi, S.
388090

FILE 'CAPLUS' ENTERED AT 09:43:38 ON 22 MAR 2001
L4 1688 SEA FILE=CAPLUS ABB=ON PLU=ON NEISSERIA(S)(POLYPEPTIDE OR PEPTIDE OR PROTEIN OR POLYPROTEIN)
L5 609 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (DNA OR DEOXYRIBONCULEIC OR DEOXY RIBONUCLEIC OR NUCLEOTIDE)
L6 19 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND SDS(W)(PAGE OR (POLYACRYL? OR POLY ACRYL?)(1W)ELECTROPHOR?)

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L6 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:569562 CAPLUS
DOCUMENT NUMBER: 133:292765
TITLE: Two glycosylase/abasic lyases from Neisseria mucosa that initiate **DNA** repair at sites of UV-induced photoproducts
AUTHOR(S): Nyaga, Simon G.; Lloyd, R. Stephen
CORPORATE SOURCE: Center for Molecular Science, The University of Texas Medical Branch, Galveston, TX, 77555, USA
SOURCE: J. Biol. Chem. (2000), 275(31), 23569-23576
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Diverse organisms ranging from Escherichia coli to humans contain a variety of **DNA** repair proteins that function in the removal of damage caused by shortwave UV light. This study reports the identification, purifn., and biochem. characterization of two **DNA** glycosylases with assocd. abasic lyase activity from Neisseria mucosa. These enzymes, pyrimidine dimer glycosylase I and II (Nmu-pdg I and Nmu-pdg II), were purified 30,000- and 10,000-fold, resp. **SDS-poly-acrylamide** gel **electrophoresis** anal. indicated that Nmu-pdg I is approx. 30 kDa, whereas Nmu-pdg II is approx. 19 kDa. The N-terminal amino acid sequence of Nmu-pdg II exhibits 64 and 66% identity with E. coli and Hemophilus parainfluenzae endonuclease III, resp. Both Nmu-pdg I and Nmu-pdg II were found to have broad substrate specificities, as evidenced by their ability to incise **DNA** contg. many types of UV and some types of oxidative damage. Consistent with other glycosylase/abasic lyases, the existence of a covalent enzyme-**DNA** complex could be demonstrated for both Nmu-pdg I and II when reactions were carried out in the presence of sodium borohydride. These data indicate the involvement of an amino group in the catalytic reaction mechanism of both enzymes.
REFERENCE COUNT: 33
REFERENCE(S): (2) Bailly, V; Biochemistry 1987, V242, P565
CAPLUS

Searcher : Shears 308-4994

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(4) Cunningham, R; Ann N Y Acad Sci 1994, V726, P215 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:277031 CAPLUS
DOCUMENT NUMBER: 133:172879
TITLE: Genetic characterization of pilin glycosylation in Neisseria meningitidis
AUTHOR(S): Power, Peter M.; Roddam, Louise F.; Dieckelmann, Manuela; Srikhanta, Yogitha N.; Tan, Yoke Cheng; Berrington, Andrew W.; Jennings, Michael P.
CORPORATE SOURCE: Department of Microbiology and Parasitology, The University of Queensland, Brisbane, 4072, Australia
SOURCE: Microbiology (Reading, U. K.) (2000), 146(4), 967-979
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pili of Neisseria meningitidis are a key virulence factor, being the major adhesin of this capsulate organism and contributing to specificity for the human host. Pili are post-translationally modified by addn. of an O-linked trisaccharide, Gal(.beta.1-4)Gal(.alpha.1-3)2,4-diacetimido-2,4,6-trideoxyhexose. In a previous study the authors identified and characterized a gene, pglA, encoding a galactosyltransferase involved in pilin glycosylation. In this study a set of random genomic sequences from N. meningitidis strain MC58 was used to search for further genes involved in pilin glycosylation. Initially, an open reading frame was identified, and designated pglD (pilin glycosylation gene D), which was homologous to genes involved in polysaccharide biosynthesis. The region adjacent to this gene was cloned and **nucleotide** sequence anal. revealed two further genes, pglB and pglC, which were also homologous with genes involved in polysaccharide biosynthesis. Insertional mutations were constructed in pglB, pglC and pglD in N. meningitidis C311#3, a strain with well-defined LPS and pilin-linked glycan structures, to det. whether these genes had a role in the biosynthesis of either of these mols. Anal. of these mutants revealed that there was no alteration in the phenotype of LPS in any of the mutant strains as judged by **SDS-PAGE** gel migration. In contrast, increased

gel migration of the pilin subunit mols. of pglB, pglC and pglD mutants by Western anal. was obsd. Pilin from each of the pglB, pglC and pglD mutants did not react with a terminal-galactose-specific stain, confirming that the gel migration differences were due to the alteration or absence of the pilin-linked trisaccharide structure in these mutants. In addn., antisera specific for the C311#3 trisaccharide failed to react with pilin from the pglB, pglC, pglD and galE mutants. Anal. of **nucleotide** sequence homologies has suggested specific roles for pglB, pglC and pglD in the biosynthesis of the 2,4-diacetimido-2,4,6-trideoxyhexose structure.

REFERENCE COUNT: 60

REFERENCE(S):
(2) Allen, A; Mol Microbiol 1996, V19, P37 CAPLUS
(3) Altschul, S; Nucleic Acids Res 1997, V25, P3389 CAPLUS
(4) Annunziato, P; J Bacteriol 1995, V177, P312 CAPLUS
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(7) Bechthold, A; Mol Gen Genet 1995, V248, P610 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:53852 CAPLUS
DOCUMENT NUMBER: 132:106952
TITLE: Screening of Neisseria vaccine candidates and vaccines against pathogenic neisseria
INVENTOR(S): Ala'aldeen, Dlawer; Todd, Ian
PATENT ASSIGNEE(S): The University of Nottingham, UK
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000003003	A2	20000120	WO 1999-GB2205	19990709
WO 2000003003	A3	20000413		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE,

DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9947917 A1 20000201 AU 1999-47917 19990709

PRIORITY APPLN. INFO.: GB 1998-14902 19980710
WO 1999-GB2205 19990709

AB Methods of screening for vaccine candidates, vaccines against pathogenic neisseria and intermediaries for such vaccines have been developed. Two vaccine candidates TspA and TspB have been identified and characterized which either alone or in conjunction with the vaccines provide for treatment against pathogenic neisserias in particular Neisseria meningitidis and/or Neisseria gonorrhoeae.

L6 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:682300 CAPLUS

DOCUMENT NUMBER: 132:32985

TITLE: Neisseria gonorrhoeae bacterioferritin: structural heterogeneity, involvement in iron storage and protection against oxidative stress

AUTHOR(S): Chen, Cheng-Yen; Morse, Stephen A.

CORPORATE SOURCE: Division of AIDS, Sexually Transmitted Diseases and Tuberculosis Laboratory Research, Centers for Disease Control and Prevention, National Centers for Infectious Disease, Atlanta, GA, 30333, USA

SOURCE: Microbiology (Reading, U. K.) (1999), 145(10), 2967-2975
CODEN: MROBEO; ISSN: 1350-0872

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The iron-storage **protein** bacterioferritin (Bfr) from **Neisseria** gonorrhoeae strain F62 was identified in cell-free exts. and subsequently purified by column chromatog. Gonococcal Bfr had an estd. mol. mass of 400 kDa by gel filtration; however, anal. by **SDS-PAGE** revealed that it was composed of 18 kDa (BfrA) and 22 kDa (BfrB) subunits. **DNA** encoding BfrB was amplified by PCR using degenerate primers derived from the N-terminal amino acid sequence of BfrB and from a C-terminal amino acid sequence of Escherichia coli Bfr. The **DNA** sequence of bfrA was subsequently obtained by genome walking using single-specific-primer PCR. The two Bfr genes were located in tandem with an intervening gap of 27 bp. A potential Fur-binding sequence (12 of 19 bp identical to the consensus neisserial fur sequence) was located within the 5' flanking region of bfrA in front of a putative -35 hexamer. The homol. between the **DNA** sequences of bfrA and bfrB was 55.7%; the deduced amino acid sequences of BfrA (154 residues) and BfrB (157 residues) showed 39.7% identity, and showed 41.3% and 56.1% identity, resp., to E.

coli Bfr. Expression of recombinant BfrA and BfrB in E. coli strain DH5.alpha. was detected on Western blots probed with polyclonal anti-E. coli Bfr antiserum. Most Bfrs are homopolymers with identical subunits; however, the evidence presented here suggests that gonococcal Bfr was composed of two similar but not identical subunits, both of which appear to be required for the formation of a functional Bfr. A Bfr-deficient mutant was constructed by inserting the .OMEGA. fragment into the BfrB gene. The growth of the BfrB-deficient mutant in complex medium was reduced under iron-limited conditions. The BfrB-deficient mutant was also more sensitive to killing by H2O2 and paraquat than the isogenic parent strain. These results demonstrate that gonococcal Bfr plays an important role in iron storage and protection from iron-mediated oxidative stress.

REFERENCE COUNT: 46

REFERENCE(S):
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(5) Braun, V; Biol Chem 1997, V378, P779 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:624824 CAPLUS
DOCUMENT NUMBER: 127:315311
TITLE: The AroQ and PheA domains of the bifunctional P-protein from Xanthomonas campestris in a context of genomic comparison
AUTHOR(S): Gu, Wei; Williams, Donna S.; Aldrich, Henry C.; Xie, Gary; Gabriel, Dean W.; Jensen, Roy A.
CORPORATE SOURCE: Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA
SOURCE: Microb. Comp. Genomics (1997), 2(2), 141-158
CODEN: MCGEFP; ISSN: 1090-6584
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The gene (denoted aroQp.cntdot.pheA) encoding the bifunctional P-protein (chorismate mutase-P/prephenate dehydratase) from Xanthomonas campestris was cloned. AroQp.cntdot.pheA is essential for L-phenylalanine biosynthesis. **DNA** sequencing of the smallest subclone capable of functional complementation of an Escherichia coli phenylalanine auxotroph revealed a putative open reading frame (ORF) of 1200 bp that would encode a 43,438-Da protein. AroQp.cntdot.PheA exhibited 51% amino acid identity with a

Pseudomonas stutzeri homolog and greater than 30% identities with AroQp.cntdot.PheA **proteins** from Haemophilus influenzae, **Neisseria** gonorrhoeae, and a no. of enteric bacteria. AroQp.cntdot.PheA from X. campestris, when expressed in E. coli, possesses a 40-residue amino-terminal extension that is lysine-rich and that is absent in all of the AroQp.cntdot.PheA homologues known at present. About 95% of AroQp.cntdot.PheA was particulate and readily sedimented by low-speed centrifugation. Sol. prepns. of cloned AroQp.cntdot.PheA exhibited a native mol. mass of 81,000 Da, indicating that the active enzyme species is a homodimer. These prepns. were unstable after purifn. of about 40-fold, even in the presence of glycerol, which was an effective protectant before fractionation. When AroQp.cntdot.PheA was overproduced by a T7 translation vector, unusual inclusion bodies having a macromol. structure consisting of protein fibrils were obsd. by electron microscopy. Insol. protein collected at low-speed centrifugation possessed high catalytic activity. The single band obtained via **SDS-PAGE** was used to confirm the translational start via N-terminal amino acid sequencing. A perspective on the evolutionary relationships of monofunctional AroQ and PheA proteins and the AroQp.cntdot.PheA family of proteins is presented. A serC gene located immediately upstream of X. campestris aroQp.cntdot.pheA appears to reflect a conserved gene organization, and both may belong to a single transcriptional unit.

L6 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:43957 CAPLUS
DOCUMENT NUMBER: 126:100085
TITLE: Analysis of the icsBA locus required for biosynthesis of the inner core region from Neisseria meningitidis lipopolysaccharide
AUTHOR(S): van der Ley, Peter; Kramer, Marco; Martin, Adele; Richards, James C.; Poolman, Jan T.
CORPORATE SOURCE: Laboratory of Vaccine Development and Immune Mechanisms, National Institute of Public Health and the Environment, Bilthoven, 3720 BA, Neth.
SOURCE: FEMS Microbiol. Lett. (1997), 146(2), 247-253
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB By deletion mutagenesis in the entire meningococcal chromosome, we have previously identified the icsA gene, which encodes the glycosyltransferase required for adding GlcNAc to Hep-II in the inner core of meningococcal LPS. This gene has homol. to several LPS glycosyltransferases, notably to rfaK from Salmonella typhimurium and bplH from Bordetella pertussis, both of which encode GlcNAc transferases. Directly upstream of icsA is an ORF showing significant homol. to the hypothetical protein HI0653 from the

Haemophilus influenzae genome sequence, and to a lesser degree to putative glycosyltransferases from Streptococcus thermophilus and Yersinia enterocolitica. Insertional inactivation of this ORF resulted in a meningococcal strain with truncated LPS. We have named this new LPS-involved gene icsB. Differences in binding of monoclonal antibodies and in mobility on Tricine-**SDS-PAGE** showed that LPS from icsA and icsB mutants is similar but not identical. On the basis of these results, we postulated that the new gene encodes the glycosyltransferase required for adding Glc to Hep-I. Structural anal. of purified mutant LPS by electrospray mass spectrometry was used to verify this hypothesis. The compn. detd. for icsA and icsB is lipid-A-(KDO)2-(Hep)2.PEA and lipid-A-(KDO)2-(Hep)2.PEA-GlcNAc, resp. The icsA and icsB genes thus form an operon encoding the glycosyltransferases required for chain elongation from the lipid-A-(KDO)2-(Hep)2 basal structure, with IcsA first adding GlcNAc to Hep-II and IcsB subsequently adding Glc to Hep-I. Only then is completion of the lacto-N-neotetraose structure possible through the action of the lgtA-E genes.

L6 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:460738 CAPLUS
DOCUMENT NUMBER: 125:134350
TITLE: Cloning, complementation, and characterization of an rfaE homolog from Neisseria gonorrhoeae
AUTHOR(S): Levin, James C.; Stein, Daniel C.
CORPORATE SOURCE: Department Microbiology, University Maryland, College Park, MD, 20742, USA
SOURCE: J. Bacteriol. (1996), 178(15), 4571-4575
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Neisseria gonorrhoeae WS1 is a spontaneous pyocin (a bacteriocin produced by Pseudomonas aeruginosa)-resistant mutant of N. gonorrhoeae FA19 that produces a truncated lipooligosaccharide (LOS) and is nontransformable. The LOS-specific mutation in WS1 was moved into a transformable background by transforming FA19 with chromosomal **DNA** from WS1 (generating strain JWS-1). A clone (pJCL2) capable of restoring JWS-1 to wild-type LOS expression, as detected by its acquisition of reactivity with monoclonal antibodies and by its complemented **SDS-PAGE** profile, was isolated. Sequential unidirectional deletion and **DNA** sequence anal. of pJCL2 identified an open reading frame, designated lsi-7, that could complement the defect in JWS-1. Homol. searches against various databases indicated that lsi-7 had homol. with several Escherichia coli genes involved in the phosphorylation of sugars. Lsi-7 is adjacent to the lsi-6 gene, another gene involved in LOS biosynthesis. Complementation studies using Salmonella typhimurium lipopolysaccharide mutants showed lsi-6 and lsi-7 to be gonococcal

homologs of S. typhimurium rfaD and rfaE, resp. Reverse transcriptase PCR anal. demonstrated that lsi-6 and lsi-7 are part of the same transcriptional unit.

L6 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:176101 CAPLUS
DOCUMENT NUMBER: 124:252269
TITLE: Identification of a locus involved in meningococcal lipopolysaccharide biosynthesis by deletion mutagenesis
AUTHOR(S): van der Ley, Pete; Kramer, Marco; Steeghs, Liana; Kuipers, Betsy; Andersen, Svein R.; Jennings, Michael P.; Moxon, E. Richard; Poolman, Jan T.
CORPORATE SOURCE: Lab. Vaccine Development and Immune Mechanisms, Natl. Inst. Public Health and Environmental Protection, Bilthoven, 3720 BA, Neth.
SOURCE: Mol. Microbiol. (1996), 19(5), 1117-25
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel method for insertion/deletion mutagenesis in meningococci was devised. This consisted of ligating a digest of total chromosomal **DNA** to a 1.1 kb restriction fragment contg. an erythromycin-resistance marker (ermC), and subsequent transformation of the ligation mixt. into the homologous meningococcal strain H44/76. Southern blotting of a no. of the resulting erythromycin-resistant transformants demonstrated that all carried the ermC gene inserted at different positions in the chromosome. Mutants with a specific phenotype were identified by screening with the anti-lipopolysaccharide (LPS) monoclonal antibody MN4A8B2, which is specific for immunotype L3. In this way, two independent L3-neg. mutant strains were isolated. In transformation expts. with chromosomal **DNA** from these mutants, erythromycin-resistance and lack of MN4A8B2 reactivity were always linked, showing that the insertion/deletion was in a locus involved in LPS biosynthesis. On **SDS-PAGE**, the mutant LPS displayed an electrophoretic mobility intermediate between that produced by the previously isolated galE and rfaF mutant strains. Chem. anal. of the mutant LPS revealed that the structure was probably lipid A-(KDO)2-(Hep)2. Chromosomal **DNA** flanking the ermC insertion in these two mutant strains was cloned, and used as probe for the isolation of the corresponding region of the wild-type strain. From hybridization and polymerase chain reaction (PCR) anal., it could be concluded that both mutations map to the same locus. The affected gene probably encodes the glycosyltransferase necessary for adding N-acetylglucosamine to heptose.

L6 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:93922 CAPLUS
DOCUMENT NUMBER: 124:167034
TITLE: Identification and characterization of genes required for post-translational modification of Campylobacter coli VC167 flagellin
AUTHOR(S): Guerry, Patricia; Doig, Peter; Alm, Richard A.; Burr, Donald H.; Kinsella, Niamh; Trust, Trevor J.
CORPORATE SOURCE: Enteric Diseases Program, Naval Medical Res. Inst., Bethesda, MD, 20889, USA
SOURCE: Mol. Microbiol. (1996), 19(2), 369-78
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two genes have been identified in Campylobacter coil VC167 which are required for the biosynthesis of posttranslational modifications on flagellin proteins. The ptmA gene encodes a protein of predicted Mr 28486 which shows significant homol. to a family of alc. dehydrogenases from a variety of bacteria. The ptmB gene encodes a **protein** of predicted Mr 26598 with significant homol. to CMP-N-acetylneuraminic acid synthetase enzymes involved in sialic acid capsular biosynthesis in **Neisseria** meningitidis and Escherichia coli K1. Site-specific mutation of either ptmA or ptmB caused loss of reactivity with antisera specific to the post-translational modifications and a change in the isoelec. focusing fingerprints relative to the parent strains. Mutation of ptmB, but not of ptmA, caused a change in apparent Mr of the flagellin subunit is **SDS-PAGE** gels. The ptmA and ptmB genes are present in other strains of Campylobacter. In a rabbit model the ptmA mutant showed a reduced ability to elicit protection against subsequent challenge with heterologous strains of the same Lior serotype compared to the parental wild-type strain. This suggests that the surface-exposed post-translational modifications may play a significant role in the protective immune response.

L6 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:5055 CAPLUS
DOCUMENT NUMBER: 124:108167
TITLE: Molecular analysis of a locus for the biosynthesis and phase-variable expression of the lacto-N-neotetraose terminal lipopolysaccharide structure in Neisseria meningitidis
AUTHOR(S): Jennings, Michael P.; Hood, Derek, W.; Peak, Ian R. A.; Virji, Mumtaz; Moxon, E. Richard
CORPORATE SOURCE: Molecular Infectious Diseases Group and Department of Paediatrics, Institute of

Molecular Medicine, Headington, OX3 9DU, UK
SOURCE: Mol. Microbiol. (1995), 18(4), 729-40
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lipopolysaccharide (LPS) is a major determinant of Neisseria meningitidis virulence. A key feature of meningococcal LPS is the phase-variable expression of terminal structures which are proposed to have disparate roles in pathogenesis. In order to identify the biosynthetic genes for terminal LPS structures and the control mechanisms for their phase-variable expression, the lic2A gene, which is involved in LPS biosynthesis in Haemophilus influenzae, was used as a hybridization probe to identify a homologous gene in N. meningitidis strain MC58. The homologous region of **DNA** was cloned and **nucleotide** sequence anal. revealed three open reading frames (ORFs), two of which were homologous to the H. influenzae lic2A gene. All three ORFs were mutagenized by the insertion of antibiotic-resistance cassettes and the LPS from these mutant strains was analyzed to det. if the genes had a role in LPS biosynthesis. Immunol. and tricine-**SDS-PAGE** anal. of LPS from the mutant strains indicated that all three genes were probably transferases in the biosynthesis of the terminal lacto-N-neotetraose structure of meningococcal LPS. The first ORF of the locus contains a homopolymeric tract of 14 guanosine residues within the 5'-end of the coding sequence. As the lacto-N-neotetraose structure in meningococcal LPS is subject to phase-variable expression, colonies that no longer expressed the terminal structure, as detd. by monoclonal antibody binding, were isolated. Anal. of an "off" phase variant revealed a change in the no. of guanosine residues resulting in a frameshift mutation, indicating that slipped-strand mispairing mechanism, operating in the first ORF, controls the phase-variable expression of lacto-N-neotetraose.

L6 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:545656 CAPLUS
DOCUMENT NUMBER: 123:193240
TITLE: A mutation in the Neisseria gonorrhoeae rfaD homolog results in altered lipo-oligosaccharide expression
AUTHOR(S): Drazek, E. Susan; Stein, Daniel C.; Deal, Carolyn D.
CORPORATE SOURCE: Dep. Bacterial Diseases, Walter Reed Army Inst. Res., Washington, DC, 20307-5100, USA
SOURCE: J. Bacteriol. (1995), 177(9), 2321-7
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gonococcal lsi-6 locus was cloned and shown by **DNA**

sequence anal. to have homol. with the E. coli rfaD gene, which encodes ADP-L-glycero-D-mannoheptose epimerase. This enzyme is involved in the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D-mannoheptose. A site-directed frameshift mutation in lsi-6 was constructed by PCR amplification and introduced into the chromosome of Neisseria gonorrhoeae MS11 P+ by transformation. The lipo-oligosaccharides (LOS) of mutant and parental strains were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**). The lsi-6 mutant produced LOS components with apparent mol. masses of 2.6 and 3.6 kDa as compared with a 3.6-kDa band of the MS11 P+ strain. The parental LOS phenotype was expressed when a revertant was constructed by transformation of the cloned wild-type gene into the lsi-6 mutant. The immunoreactivity of LOS from parental and constructed strains was examd. by **SDS-PAGE** and Western blotting. Only the parental and reconstructed wild-type strains produced a 3.6-kDa LOS component that reacted with monoclonal antibody 2-1-L8. These results suggest that the lsi-6 locus is involved in gonococcal LOS biosynthesis and that the nonreactive mutant 3.6-kDa LOS component contains a conformational change or altered saccharide compn. that interferes with immunoreactivity.

L6 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:	1995:292406 CAPLUS
DOCUMENT NUMBER:	122:124946
TITLE:	Lipooligosaccharide biosynthesis in Neisseria gonorrhoeae: Cloning, identification and characterization of the .alpha.1,5 heptosyltransferase I gene (rfaC)
AUTHOR(S):	Zhou, Daoguo; Lee, Na-Gyong; Apicella, Michael A.
CORPORATE SOURCE:	Department Microbiology, University Iowa, Iowa City, IA, 52242, USA
SOURCE:	Mol. Microbiol. (1994), 14(4), 609-18 CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The identical partial deep-core structure of Hep.alpha.1-3Hep.alpha.1-5KDO in Salmonella typhimurium LT2 LPS and Neisseria gonorrhoeae LOS enabled us to isolate a **DNA** fragment from N. gonorrhoeae that was able to complement the .alpha.1,5 LOS heptosyltransferase defect in the S. typhimurium rfaC630 (SA1377) mutant. **SDS-PAGE** anal. confirmed the prodn. of wild-type LPS in the transformant. Subcloning revealed that complementation was due to a 1.2 kb fragment. Sequence anal. revealed a complete open reading frame capable of encoding a 36-37 kDa peptide. In vitro transcription-translation anal. of the 1.2 kb clone confirmed that a 37 kDa protein was encoded by this **DNA** fragment. The **DNA** sequence-deduced protein

had 36% identity and 58% similarity to S. typhimurium heptosyltransferase I (RfaC). Primer extension anal. indicated that transcription of the cloned gene in N. gonorrhoeae strain 1291 begins 144 bp upstream of the start codon at a G **nucleotide** . An isogenic mutant of N. gonorrhoeae strain 1291 with an m-Tn3 insertion inside the coding sequence expressed a single truncated LOS with a similar mol. mass to S. typhimurium rfaC LPS. We conclude that the 1.2 kb fragment encodes the .alpha.1,5 LOS heptosyltransferase I (RfaC) in N. gonorrhoeae. Our studies also provide further evidence that the third KDO residue in S. typhimurium LPS is added after the core synthesis is completed.

L6 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:107608 CAPLUS
DOCUMENT NUMBER: 122:234202
TITLE: Genetic locus for the biosynthesis of the variable portion of Neisseria gonorrhoeae lipooligosaccharide
AUTHOR(S): Gotschlich, Emil C.
CORPORATE SOURCE: Lab. Bacterial Pathogenesis Immunol., Rockefeller Univ., New York, NY, 10021-6399, USA
SOURCE: J. Exp. Med. (1994), 180(6), 2181-90
CODEN: JEMEAV; ISSN: 0022-1007
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A locus involved in the biosynthesis of gonococcal lipooligosaccharide (LOS) has been cloned from gonococcal strain F62. The locus contain five open reading frames. The first and second reading frames are homologous, but not identical, to the fourth and fifth reading frames, resp. Interposed is an addnl. reading frame which has distant homol. to the Escherchia coli rfaI and rfaJ genes, both glucosyl transferases involved in lipopolysaccharide core biosynthesis. The second and fifth reading frames show strong homol. to the lex-1 of lic2A gene of Haemophilus influenzae, but do not contain the CAAT repeats found in this gene. Deletions of each of these five genes, of combinations of genes, and of the entire locus were constructed and introduced into parental gonococcal strain F62 by transformation. The LOS phenotypes were then analyzed by **SDS-PAGE** and reactivity with monoclonal antibodies. Anal. of the gonococcal mutants indicates that four of these genes are the glycosyl transferases that add GalNAc.beta.1 .fwdarw. 3Gal.beta.1 .fwdarw. 4GalNAc.beta.1 .fwdarw. 3Gal.beta.1 .fwdarw. 4 to the substrate Glc.beta.1 .fwdarw. 4Hep .fwdarw. R of the inner core region. The gene with homol. to E. coli rfaI/rfaJ is involved with the addn. of the .alpha.-linked galactose residue in the biosynthesis of the alternative LOS structure Gal.alpha.1 .fwdarw. Gal.beta.1 .fwdarw. 4Gal.beta.1 .fwdarw. 4Hep .fwdarw. R. Since these genes encode LOS glycosyl transferases they have been named lgtA, lgtB, lgtC, lgtD, and lgtE.

The **DNA** sequence anal. revealed that lgtA, lgtC, and lgtD contained poly-G tracts, which, in strain F62 were, resp., 17, 10, and 11 bp. Thus, three of the LOS biosynthetic enzymes are potentially susceptible to premature termination by reading frame changes. It is likely that these structural features are responsible for the high-frequency genetic variation of gonococcal LOS.

L6 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:404768 CAPLUS
DOCUMENT NUMBER: 121:4768
TITLE: Lipooligosaccharide biosynthesis in pathogenic Neisseria. Cloning, identification, and characterization of the phosphoglucomutase gene
AUTHOR(S): Zhou, Daoguo; Stephens, David S.; Gibson, Bradford W.; Engstrom, Jeffrey J.; McAllister, Carl F.; Lee, Frank K. N.; Apicella, Michael A.
CORPORATE SOURCE: Dep. Microbiol., Univ. Iowa, Iowa City, IA, 52242, USA
SOURCE: J. Biol. Chem. (1994), 269(15), 11162-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lipooligosaccharide (LOS) of pathogenic Neisseria is an important factor in disease pathogenesis. Little is known about the genes involved in neisserial LOS biosynthesis. To elucidate specific LOS biosynthetic genes, the authors screened a Tn916 library that was constructed in Neisseria meningitidis strain NMB. This strain expresses a single LOS that has an mol. mass of 4.5 kDa and binds monoclonal antibody (mAb) 3F11. This library was screened using a mAb panel that recognizes structural differences in neisserial LOS oligosaccharides. A stable LOS mutant of strain NMB was identified which the authors designated NMB-R6. This mutant expressed an LOS with an mol. mass of approx. 3.1-3.2 kDa and did not bind mAb 3F11. Genomic **DNA** from this mutant transformed N. meningitidis strain NMB to the tetracycline resistant NMB-R6 phenotype greater than 10-4/recipient/.mu.g of **DNA**. In addn., the authors transformed Neisseria gonorrhoeae strain 1291 (LOS phenotype mol. mass 4.5 kDa, mAb 3F11+) to the NMB-R6 LOS phenotype with N. meningitidis NMB-R6 genomic **DNA**. Anal. of N. gonorrhoeae strain 1291-R6 LOS by mass spectroscopy showed that the LOS oligosaccharide structure is GlcNAc .fwdarw. Hep2phosphoethanolamine .fwdarw. 2-keto-3-deoxymannooctulosonic acid (where Hep is heptose). Sequence anal. showed that the transposon is inserted into the 3' end of a gene that has homol. to the human phosphoglucomutase (PGM) gene. Sequence comparison indicated that the putative PGM gene in N. gonorrhoeae 1291 and N. meningitidis NMB had 92% identity at the **DNA** level. PGM and glucokinase activity was present in cell free exts. of N. meningitidis NMB and

N. gonorrhoeae strain 1291. N. meningitidis NMB-R6 and N. gonorrhoeae strain 1291-R6 had no detectable PGM activity, whereas glucokinase activity was similar to the wild type strains. PGM activity can be reconstituted in N. meningitidis strain NMB-R6 by transformation with the cloned PGM gene. **SDS-polyacrylamide** gel **electrophoresis** demonstrated that NMB-R6 transformed with the PGM gene expressed the 3F11+, 4.5-kDa LOS of the parent NMB strain. The inability of N. meningitidis NMB-R6 and N. gonorrhoeae strain 1291-R6 to convert glucose 6-phosphate to glucose 1-phosphate results in the truncated LOS phenotype expressed by these mutants.

L6 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:212128 CAPLUS
DOCUMENT NUMBER: 120:212128
TITLE: Pilus-facilitated adherence of Neisseria meningitidis to human epithelial and endothelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin
AUTHOR(S): Virji, Mumtaz; Saunders, Jon R.; Sims, Gail; Makepeace, Katherine; Maskell, Duncan; Ferguson, David J. P.
CORPORATE SOURCE: Dep. Paediatr., Univ. Oxford, Oxford, OX3 9DU, UK
SOURCE: Mol. Microbiol. (1993), 10(5), 1013-28
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adherence of capsulate N. meningitidis to endothelial and epithelial cells is facilitated in variants that express pili. Whereas piliated variants of N. meningitidis strain C311 adhered to endothelial cells in large nos. (>150 bacteria/cell), derivs. contg. specific mutations that disrupt pilE encoding the pilin subunit were both non-piliated and failed to adhere to endothelial cells (<1 bacterium/cell). In addn., meningococcal pili recognized human endothelial and epithelial cells but not cells originating from other animals. Variants of strain C311 were obtained that expressed pilins of reduced apparent Mr and exhibited a marked increase in adherence to epithelial cells. Structural anal. of pilins from 2 hyper-adherent variants and the parent strain were carried out by **DNA** sequencing of their pilE genes. Deduced mol. wts. of pilins were considerably lower than their apparent Mr values on **SDS-PAGE**. Hyperadherent pilins shared unique changes in sequence, including substitution of Asn-113 for Asp-113 and changes from Asn-Asp-Thr-Asp to Thr-Asp-Ala-Lys at residues 127-130 in mature pilin. Asn residues 113 and 127 of parental pilin both form part of the typical eukaryotic N-glycosylation motif Asn-X-Ser/Thr and could potentially be glycosylated

post-translationally. The presence of carbohydrate on pilin was demonstrated and when pilins were deglycosylated, their migration on **SDS-PAGE** increased, supporting the notion that variable glycosylation accounts for discrepancies in apparent and deduced mol. wts. Functionally distinct pilins produced by 2 fully piliated variants of a 2nd strain (MC58) differed only in that the putative glycosylation motif Asn-60-Asn-61-Thr-62 in an adherent variant was replaced with Asp-60-Asn-61-Ser-62 in a nonadherent variant. Fully adherent backswitchers obtained from the nonadherent variant always regained Asn-60 but retained Ser-62. It is proposed, therefore, that functional variations in N. meningitidis pili may be modulated in large part by primary amino acid sequence changes that ablate or create N-linked glycosylation sites on the pilin subunit.

L6 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:28863 CAPLUS
DOCUMENT NUMBER: 120:28863
TITLE: Expression of meningococcal epitopes in LamB of Escherichia coli and the stimulation of serosubtype-specific antibody responses
AUTHOR(S): McCarvil, J.; McKenna, A. J.; Grief, C.; Hoy, C. S.; Sesardic, D.; Maiden, M. C. J.; Feavers, I. M.
CORPORATE SOURCE: Div. Bacteriol., Natl. Inst. Biol. Stand. and Control, South Mimms/Potters Bar, EN6 3QG, UK
SOURCE: Mol. Microbiol. (1993), 10(1), 203-13
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The class 1 outer membrane **protein** (OMP), a major variable surface antigen of **Neisseria** meningitidis, is a component of novel meningococcal vaccines currently in field trials. Serol. variants of the protein are also used to serosubtype meningococci. Most of the amino acid changes that give rise to antigenic variants of the protein occur in 2 variable regions (VR1 and VR2) that are thought to form loops on the cell surface. The polymerase chain reaction. (PCR) was used to amplify the **nucleotide** sequences encoding VR1 and VR2 from the chromosomal **DNA** of N. meningitidis strain M1080. These were cloned in frame into the LamB gene of the Escherichia coli expression vector pAJC264. Whole-cell ELISAs, using monoclonal antibodies, and **SDS-PAGE** confirmed that, upon induction, strains of E. coli carrying these constructs expressed hybrid LamB proteins contg. the N. meningitidis surface loops. These strains were used to immunize rabbits and the resultant polyclonal antisera reacted specifically with the class I OMP of ref. strain M1080 (P1.7). Immunogold labeling of meningococcal cells and whole-cell dot-blot analyses with these antisera showed that the variable epitopes were exposed on the cell surface and confirmed that this approach could be used

to obtain serosubtype-specific antisera. The binding profiles of the antisera were detd. from their reactions with overlapping synthetic peptides and their reactivity compared with that of relevant serosubtype-specific monoclonal antibodies. This approach was used successfully to raise antisera against 2 other class 1 OMP VR2s. A fourth antiserum raised against a VR2, including the P1.1 epitope, was not subtype specific.

L6 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:226653 CAPLUS
DOCUMENT NUMBER: 118:226653
TITLE: Identification of meningococcal serosubtypes by polymerase chain reaction
AUTHOR(S): Maiden, Martin C. J.; Bygraves, Jane A.; McCarvil, James; Feavers, Ian M.
CORPORATE SOURCE: Nal. Inst. Biol. Stand. Control, Hertsfordshire, EN6 3QG, UK
SOURCE: J. Clin. Microbiol. (1992), 30(11), 2835-41
CODEN: JCMIDW; ISSN: 0095-1137
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The polymerase chain reaction was used as the basis of a novel typing method for Neisseria meningitidis. Southern hybridization expts. demonstrated that it was possible to identify genes encoding different serol. variants of the meningococcal class 1 outer membrane protein by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14 defined variable regions was prepd. in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane protein genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one expt. This technique was evaluated with a set of serol. ref. strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of **SDS -polyacrylamide** gel **electrophoresis** or serol. anal. An addnl. advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the **DNA** amplified from the original sample could be used to det. the **nucleotide** sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clin. samples even when culture is not possible because of antibiotic treatment of an acute case.

L6 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1990:546610 CAPLUS
DOCUMENT NUMBER: 113:146610
TITLE: Stable expression of meningococcal class 1 protein in an antigenically reactive form in outer membranes of Escherichia coli
AUTHOR(S): White, D. A.; Barlow, A. K.; Clarke, I. N.; Heckels, J. E.
CORPORATE SOURCE: Med. Sch., Univ. Southampton, Southampton, SO9 4XT, UK
SOURCE: Mol. Microbiol. (1990), 4(5), 769-76
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The entire gene encoding the class 1 outer membrane **protein** of **Neisseria** meningitidis is located on a 2.2 kb fragment obtained on digestion of chromosomal **DNA** with XbaI. This XabaI fragment from strain MC50 (subtype P1-16), which had previously been cloned in bacteriophage M13, was transferred to the plasmid vector pMTL20. The resulting plasmid (pPORA100) was propagated in E. coli (JM109) and cell lysates were subjected to **SDS-PAGE**. Western blotting with anti-class I protein antibodies revealed constitutive expression of a protein of 41 kD, corresponding to the class 1 protein of the parent meningococcal strain, which was absent in the E. coli control. Fractionation of E. coli cells carrying the recombinant plasmid revealed that the protein exclusively located in the outer membrane, and N-terminal amino acid anal. of the expressed protein revealed that normal processing of the signal peptide had occurred. Immunogold electron microscopy showed that the protective epitope recognized by a P1-16 subtype-specific monoclonal antibody was exposed in an antigenically reactive form on the surface of E. coli cells carrying plasmid pPORA100. In contrast, expression in E. coli of a 2nd plasmid (pPORA 104) lacking the coding sequence for the 1st 15 amino acids of the signal peptide resulted in accumulation of recombinant class 1 protein only in the cytoplasm of the cells. Thus, the presence of the meningococcal signal sequence ensures expression of this meningococcal porin protein in an antigenically native conformation in outer membrane of E. coli, while its absence results in expression of a sol. protein. Such constructs illustrate the potential use of recombinant **DNA** technol. for the development of effective human vaccines against meningococcal infection.

L6 ANSWER 19 OF 19 CAPLUS
ACCESSION NUMBER: 1982:488519 CAPLUS
DOCUMENT NUMBER: 97:88519
TITLE: Genetic linkage between serogroup specificity and antibiotic resistance in Neisseria gonorrhoeae

AUTHOR(S): Bygdeman, Solgun; Baeckman, Marianne; Danielsson, Dan; Norgren, Mari

CORPORATE SOURCE: Dep. Clin. Bacteriol., Karolinska Inst., Stockholm, Swed.

SOURCE: Acta Pathol., Microbiol. Immunol. Scand., Sect. B (1982), 90B(3), 243-50
CODEN: APMMD2

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Significant differences were demonstrated in antibiotic susceptibility between gonococcal strains of the recently described W serogroups, W I, W II, and W III. Strains of serogroup W I were almost always sensitive to penicillin and other antibiotics, whereas those of the W II and W III serogroups showed a higher incidence of decreased susceptibility. Transformation expts. were undertaken with an antibiotic sensitive serogroup W I gonococcal strain as recipient and a multiresistant W II strain as **DNA**-donor. Transformants with increased resistance to penicillin and several other antibiotics as compared with the recipient acquired the same serogroup specificity as the W II donor. With one of these W II transformants as donor and the sensitive W I strain as recipient, all transformants acquired the same antibiotic susceptibility pattern and serogroup as the donor. **SDS-polyacrylamide** gel **electrophoresis**, performed on Sarkosyl-extd. outer membrane proteins from donor, recipient, and some transformants, showed an increase in the mol. wt. of the Protein I of the outer membrane of the W II transformants as compared with that of the recipient strain. In rocket-line and crossed-line immunoelectrophoresis the W II transformants could not be distinguished from the W II donor strain. A genetic linkage between antibiotic multiresistance and serogroup W II specificity was thus shown. This is in agreement with the demonstrated higher incidence of W II strains with increased antibiotic resistance as compared with that of serogroup W I strains.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 09:57:13 ON 22 MAR 2001)

L7 48 S L6

L8 24 DUP REM L7 (24 DUPLICATES REMOVED)

L8 ANSWER 1 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 2001:86340 BIOSIS

DOCUMENT NUMBER: PREV200100086340

TITLE: Analysis of lipooligosaccharide biosynthesis in the Neisseriaceae.

AUTHOR(S): Arking, Dan; Tong, Yanhong; Stein, Daniel C. (1)

CORPORATE SOURCE: (1) Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, 20742: DS64@UMAIL.UMD.EDU USA

SOURCE: Journal of Bacteriology, (February, 2001) Vol. 183, No. 3, pp. 934-941. print.
ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Neisserial lipooligosaccharide (LOS) contains three oligosaccharide chains, termed the alpha, beta, and gamma chains. We used Southern hybridization experiments on **DNA** isolated from various **Neisseria** spp. to determine if strains considered to be nonpathogenic possessed **DNA** sequences homologous with genes involved in the biosynthesis of these oligosaccharide chains. The presence or absence of specific genes was compared to the LOS profiles expressed by each strain, as characterized by their mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and their reactivities with various LOS-specific monoclonal antibodies. A great deal of heterogeneity was seen with respect to the presence of genes encoding glycosyltransferases in **Neisseria**. All pathogenic species were found to possess **DNA** sequences homologous with the lgt gene cluster, a group of genes needed for the synthesis of the alpha chain. Some of these genes were also found to be present in strains considered to be nonpathogenic, such as **Neisseria** lactamica, N. subflava, and N. sicca. Some nonpathogenic **Neisseria** spp. were able to express high-molecular-mass LOS structures, even though they lacked the **DNA** sequences homologous with rfaF, a gene whose product must act before gonococcal and meningococcal LOS can be elongated. Using a PCR amplification strategy, in combination with **DNA** sequencing, we demonstrated that N. subflava 44 possessed lgtA, lgtB, and lgtE genes. The predicted amino acid sequence encoded by each of these genes suggested that they encoded functional **proteins**; however, structural analysis of LOS isolated from this strain indicated that the bulk of its LOS was not modified by these gene products. This suggests the existence of an additional regulatory mechanism that is responsible for the limited expression of these genes in this strain.

L8 ANSWER 2 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-293149 [25] WPIDS
DOC. NO. CPI: C2000-088672
TITLE: Isolated outer membrane protein from a Moraxella catarrhalis strain used for diagnosis treatment and prevention of disease caused by M. catarrhalis e.g. pneumonia, otitis media and respiratory infections.
DERWENT CLASS: B04 D16
INVENTOR(S): TILLMANN, U F; TUCKER, K
PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000018910	A1	20000406	(200025)*	EN	108

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW

AU 9964100	A	20000417	(200035)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000018910	A1	WO 1999-US22918	19991001
AU 9964100	A	AU 1999-64100	19991001

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9964100	A	Based on	WO 200018910

PRIORITY APPLN. INFO: US 1998-164714 19981001

AN 2000-293149 [25] WPIDS

AB WO 200018910 A UPAB: 20000524

NOVELTY - An isolated or substantially purified outer membrane protein (OMP), OMP21, from a Moraxella catarrhalis strain with an apparent molecular weight of 16-20 kD as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis (**SDS-PAGE**), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a peptide fragment of OMP21 which specifically binds to an antibody that specifically binds OMP21;

(2) an isolated nucleic acid molecule (I) encoding OMP21, a complementary sequence, a sequence substantially homologous to, or any fragment of OMP21;

(3) plasmid pOMP21X obtainable from Escherichia coli Top10F' (pOMP21X) deposited as ATCC 98878;

(4) a recombinant expression vector (II) adapted for transformation of a host cell comprising (I) or the plasmid of (3);

(5) a recombinant expression vector (III) adapted for transformation of a host cell comprising (I) and an expression system operatively coupled to a nucleic acid molecule for expression by the host of OMP21;

(6) a transformed host cell containing (II) or (III);

(7) an isolated recombinant OMP21 producible by the transformed host cell of (6);

(8) an attenuated or inactivated cultivar of M. catarrhalis where the cultivar has been genetically manipulated to delete the nucleic acid encoding OMP21 so it is non-transcribed;

(9) a pharmaceutical composition which is prophylactic, therapeutic, or immunogenic including a vaccine, or a vaccine comprising at least one component selected from:

(a) OMP21;

(b) (I);

(c) OMP21, obtained from a transformed host comprising an expression vector containing (I) and a means of expression coupled to the nucleic acid for expression of OMP21 by the host;

(d) a recombinant vector comprising (I); and

(e) a transformed cell comprising the vector of (d);

(10) antisera raised against the compositions or vaccine of (9);

(11) an isolated antibody (IV) present in the antisera of (10) that specifically binds one or more of the components present in the compositions or vaccine of (9);

(12) a method for detecting anti-M. catarrhalis antibodies in a test sample comprising:

(a) contacting a test sample with the composition of (9) in the presence of anti-M. catarrhalis antibodies to form antigen:anti-M. catarrhalis antibody immunocomplexes; and

(b) detecting any immunocomplexes formed as an indication of the presence of anti-M. catarrhalis antibodies in the test sample;

(13) a diagnostic kit for detecting antibodies to M. catarrhalis comprising the pharmaceutical compositions of (9), a container and a reagent for detecting M. catarrhalis antigen:anti-M. catarrhalis antibody immunocomplexes formed between the compositions and the sample;

(14) a method for detecting the presence of M. catarrhalis in a test sample comprising contacting a test sample with the antibodies of (11) and detecting any immunocomplexes formed as an indication of the presence of M. catarrhalis in the test sample;

(15) a diagnostic kit for detecting the presence of M. catarrhalis comprising the antibodies of (11), a container and a reagent for measuring M. catarrhalis:anti-M. catarrhalis antibody immunocomplexes formed between the antibodies and M. catarrhalis;

(16) a method for determining the presence of nucleic acid encoding OMP21 in a sample comprising contacting a sample with (I) to produce duplexes comprising (I) and any nucleic acid molecule encoding OMP21 in the sample specifically hybridizable with (I) and detecting the duplexes produced;

(17) a diagnostic kit for determining the presence of nucleic acid encoding OMP21 in a sample comprising (I), a device for contacting (I) with a test sample and a device for detecting duplexes produced; and

(18) a method of preventing, treating or ameliorating a disorder related to M. catarrhalis in an animal in need of treatment comprising administering an effective amount of the compositions or vaccine of (9).

ACTIVITY - Antibacterial; auditory; antiinflammatory.

MECHANISM OF ACTION - Vaccine.

Pre-immune serum and anti-OMP21 antiserum was examined for activity in mediating complement killing of M. catarrhalis using the Serum Bactericidal Test described by Zollinger et al in Immune responses to Neisseria meningitis, Manual of Clinical Laboratory Immunology, 3rd ed., 347-349 with cells of M. catarrhalis strains not N. meningitis cells. The anti-OMP21 antiserum mediated complement-killing of M. catarrhalis ATCC 49143 but not of a deletion mutant of M. catarrhalis with the OMP21 gene disrupted.

USE - OMP21, its nucleic acids and antibodies can be used in prophylactic and therapeutic compositions for treating a M. catarrhalis bacterial infection, otitis media, respiratory infections, sinusitis and pneumonia (claimed). They are useful as reagents for the clinical or medical diagnosis of M. catarrhalis infections and for scientific research on the properties of pathogenicity, virulence and infectivity of M. catarrhalis and host defense mechanisms.

The antibodies, particularly those that are cytotoxic may be used in passive immunization to prevent or attenuate M. catarrhalis infections of animals e.g. humans.

Dwg.0/9

L8 ANSWER 3 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2000-256581 [22] WPIDS
CROSS REFERENCE: 2000-237782 [20]
DOC. NO. CPI: C2000-078252
TITLE: **Neisseria** meningitidis NMASP **polypeptide**, **nucleotide** sequences and antibodies, useful in vaccines against infection.
DERWENT CLASS: B04 D16
INVENTOR(S): HARRIS, A M; JACKSON, W J
PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000012535	A2	20000309	(200022)*	EN	75

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG

SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW
AU 9957894 A 20000321 (200031)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000012535	A2	WO 1999-US19663	19990901
AU 9957894	A	AU 1999-57894	19990901

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9957894	A	Based on	WO 200012535

PRIORITY APPLN. INFO: US 1998-98685 19980901
AN 2000-256581 [22] WPIDS
CR 2000-237782 [20]
AB WO 200012535 A UPAB: 20000630
NOVELTY - An isolated **Neisseria** meningitidis NMASP **polypeptide**, which has a molecular weight of about 40-55 kD, determined by sodium dodecyl sulfate (**SDS**)-**PAGE** (polyacrylamide gel electrophoresis), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a peptide fragment of NMASP;

(2) an isolated antibody that specifically binds NMASP;

(3) an antigenic composition, vaccine or pharmaceutical composition comprising NMASP or a peptide fragment or an antibody of (2);

(4) an isolated **DNA** comprising a **nucleotide** sequence encoding NMASP or its fragments;

(5) an isolated **DNA** sequence having a 153 base pair (bp) sequence given in the specification;

(6) an isolated **DNA** which comprises a **nucleotide** sequence that hybridizes under high stringency conditions to a sequence of (5);

(7) plasmid pNmAH116 obtainable from Escherichia coli Top10 pNmAH116) as deposited with the ATCC and assigned accession number 98839;

(8) a method (A) for assaying for an agent that interacts with NMASP;

(9) an antagonist which inhibits the activity or expression of NMASP; and

(10) a method for identifying compounds which interact with and inhibitor or activate an activity of NMASP, comprising contacting the polypeptide with the compound to be screened under interaction conditions and assessing the interaction, an interaction being

associated with a second component capable of providing a signal in the presence or absence of a signal generated by the interaction between the polypeptide and the compound.

ACTIVITY - Antibacterial; Anti-inflammatory.

MECHANISM OF ACTION - Vaccine.

USE - NMASP can be used in a method to produce an immune response in an animal. The sequences and antibodies are useful for protection against N. meningitidis, the most common cause of bacterial meningitidis and septicemia in infants and young adults. The antibody is a cytotoxic antibody that mediates complement killing of N. meningitidis. NMASP and NMASP-derived polypeptides may be used as ligands to detect antibodies elicited in response to N. meningitidis infections.

ADVANTAGE - Antibody generated against the NMASP **polypeptide** in an animal host will exhibit bactericidal and/or opsonic activity against many **Neisseria** meningitidis strains and thus confer broad cross-strain protection. Bactericidal and/or opsonic antibody will prevent the bacterium from infecting the host and/or enhance the clearance of the pathogen by the host's immune system.

Dwg.0/2

L8 ANSWER 4 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2000-237782 [20] WPIDS
CROSS REFERENCE: 2000-256581 [20]
DOC. NO. NON-CPI: N2000-178293
DOC. NO. CPI: C2000-072442
TITLE: Non-cytosolic NGSP **polypeptide** and polynucleotide sequence from **Neisseria** useful for diagnosis, prevention or treatment of **Neisseria** infections.
DERWENT CLASS: B04 C06 C07 D16 S03
INVENTOR(S): HARRIS, A M; JACKSON, W J
PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000012133	A1	20000309	(200020)*	EN	68
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW					
AU 9959066	A	20000321	(200031)		

APPLICATION DETAILS:

Searcher : Shears 308-4994

PATENT NO	KIND	APPLICATION	DATE
WO 2000012133	A1	WO 1999-US20070	19990901
AU 9959066	A	AU 1999-59066	19990901

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9959066	A	Based on	WO 200012133

PRIORITY APPLN. INFO: US 1998-98685 19980901
AN 2000-237782 [20] WPIDS
CR 2000-256581 [20]
AB WO 200012133 A UPAB: 20000630
NOVELTY - Isolated NGSP **polypeptide** (I) of **Neisseria** spp. but not from N. meningitidis has a molecular weight of 40-55 kD determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (**SDS-PAGE**).
The NGSP **polypeptide** is the whole, or a subunit of a non-cytosolic **protein** embedded in or located in the bacterial envelope which includes the inner membrane, outer surface and periplasmic space.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a peptide fragment (II) of (I);
(2) an antibody (III) that specifically binds (I) or a fragment of (I);
(3) an antigenic, pharmaceutical or vaccine composition comprising (I) or (II) and a carrier or diluent;
(4) a pharmaceutical composition comprising (III);
(5) an isolated **DNA** (IV) comprising a **nucleotide** sequence encoding (I), (II) or a fragment of these which has the defined sequence of 153, 1242, 1395, 69 or 46 base pairs given in the specification;
(6) an isolated **DNA** comprising a **nucleotide** sequence which hybridizes under high stringency conditions to (IV) or its complement;
(7) plasmid pTLZ-NgHtrA number 2 obtainable from Escherichia coli JM109 (pTLZ-NgHtrA number 2) (ATCC PTA-470);
(8) an antagonist which inhibits the activity or expression of (I);
(9) a method for identifying compounds which interact with, inhibit or activate an activity of (I) comprising contacting a composition comprising (I) with the candidate compound (A) to permit interaction between (A) and (I); (A) is associated with a second component capable of providing a detectable signal in response to interaction of (I) with (A) so that the presence or absence of a

signal generated from the interaction is determined; and

(10) a method for assaying for an agent that interacts with (I) which can be used as a diagnostic, prophylactic or therapeutic agent against Neisseria infection comprising:

(i) contacting a cell expressing (I) with an agent labeled with a detectable marker for a sufficient length of time to allow interaction;

(ii) washing the cells; and

(iii) detecting any marker associated with the cells indicating that the agent interacts with (I).

ACTIVITY - Antibacterial.

No biological data given.

MECHANISM OF ACTION - Vaccine.

(I) has conserved Arg-Gly-Asp and Arg-Gly-Asn groups near the C-terminus which function as adherence domains for extracellular matrix proteins. Using (I) as a vaccine produces antibodies which inhibit (I) binding to the host's cellular matrix reducing attachment and/or subsequent invasion.

USE - (I) and (II) can be used to immunize an animal and produce an immune response (claimed). (I) and (II) can be used as ligands to detect antibodies elicited in response to Neisseria infections and also as antigens or immunogens for inducing Neisseria-specific antibodies which are useful in immunoassays to detect Neisseria in biological specimens. (IV) can be used as probes to identify Neisseria in biological specimens by hybridization or polymerase chain reaction amplification. (I) can also be used in screening assays to identify agents and compounds which useful as diagnostic, prophylactic or therapeutic agents against Neisseria infection (claimed).

Dwg.0/2

L8 ANSWER 5 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2000-147612 [13] WPIDS

DOC. NO. NON-CPI: N2000-109219

DOC. NO. CPI: C2000-046316

TITLE: Generation of cell lines and clones specific to a particular protein for screening antigenic peptides which are used as vaccines in treating meningococcal, gonococcal infections.

DERWENT CLASS: B04 D16 S03

INVENTOR(S): ALA'ALDEEN, D; TODD, I

PATENT ASSIGNEE(S): (UYNO-N) UNIV NOTTINGHAM

COUNTRY COUNT: 86

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000003003	A2	20000120	(200013)*	EN	51

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC

MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK
LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG
SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9947917 A 20000201 (200028)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000003003	A2	WO 1999-GB2205	19990709
AU 9947917	A	AU 1999-47917	19990709

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9947917	A	Based on	WO 200003003

PRIORITY APPLN. INFO: GB 1998-14902 19980710
AN 2000-147612 [13] WPIDS
AB WO 200003003 A UPAB: 20000313
NOVELTY - Generation of T-cell lines and clones (C) specific to neisserial proteins (P) is new and comprises isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with (P), stimulating proliferation of T cell lines and clones and maintaining the same by regular proliferation stimulation.

DETAILED DESCRIPTION - Generation of T-cell lines and clones (C) specific to neisserial proteins (P) is new and comprises isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with (P) with or without a proliferation stimulant, stimulating proliferation of T cell lines and clones which are specific to neisserial proteins and maintaining the same by regular stimulation.

INDEPENDENT CLAIMS are also included for the following:

(1) a method (II) of detecting CD4+T-cell (T) stimulating proteins, by fractionating (P) and testing their ability to proliferate (C);

(2) a method (III) and (IV) of detecting CD4+ T cell stimulating recombinant proteins and peptides by screening genomic meningococcal or gonococcal expression library (L) and their phage display libraries (PDLs) respectively;

(3) a method of detecting CD4+T-cell stimulating peptides comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell

lines and clones;
(4) use of a polypeptide in the manufacture of a vaccine (V) against neisserial disease comprising an 880 amino acid sequence (fully defined in the specification)
(5) CD4+ stimulant polypeptide comprising 880 amino acids (aa) or 548 aa;
(6) **nucleotides** sequence comprising 2761 base pairs (bp) or 1647 bp or their active derivatives;
(7) vaccine comprising (5); and
(8) a purified and isolated **DNA** composition comprising (6).
ACTIVITY - Antibacterial; anti-inflammatory
MECHANISM OF ACTION - Vaccine.
USE - (I) is used to generate (C) which are used in (II, III and IV) to identify CD4+ T-cell stimulants. The polypeptide and composition comprising peptides having 880 aa or 548 aa are used as vaccines for treating neisserial diseases by inducing T-cell proliferation (claimed). **DNA** construct comprising 2761 bp or 1647 bp is used in the manufacture of medicament for treating neisserial diseases (claimed) like meningitis, gonorrhoea, overwhelming septicemia, septic arthritis.
ADVANTAGE - The peptide epitope that stimulates specific CD4+ T-cells is identified and characterized. The vaccine includes non-capsular antigens that stimulate T-cell memory and thus a long lasting, cross protective immunity is generated.
Dwg.0/3

L8 ANSWER 6 OF 24 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000420826 MEDLINE
DOCUMENT NUMBER: 20378984
TITLE: Two glycosylase/abasic lyases from Neisseria mucosa that initiate **DNA** repair at sites of UV-induced photoproducts.
AUTHOR: Nyaga S G; Lloyd R S
CORPORATE SOURCE: Center for Molecular Science, the University of Texas Medical Branch, Galveston, Texas 77555, USA.
CONTRACT NUMBER: ES04091 (NIEHS)
ES06676 (NIEHS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 4) 275 (31) 23569-76.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200011
ENTRY WEEK: 20001102
AB Diverse organisms ranging from Escherichia coli to humans contain a variety of **DNA** repair **proteins** that function in

the removal of damage caused by shortwave UV light. This study reports the identification, purification, and biochemical characterization of two **DNA** glycosylases with associated abasic lyase activity from **Neisseria** mucosa. These enzymes, pyrimidine dimer glycosylase I and II (Nmu-pdg I and Nmu-pdg II), were purified 30,000- and 10,000-fold, respectively. **SDS-polyacrylamide** gel **electrophoresis** analysis indicated that Nmu-pdg I is approximately 30 kDa, whereas Nmu-pdg II is approximately 19 kDa. The N-terminal amino acid sequence of Nmu-pdg II exhibits 64 and 66% identity with E. coli and Hemophilus parainfluenzae endonuclease III, respectively. Both Nmu-pdg I and Nmu-pdg II were found to have broad substrate specificities, as evidenced by their ability to incise **DNA** containing many types of UV and some types of oxidative damage. Consistent with other glycosylase/abasic lyases, the existence of a covalent enzyme-**DNA** complex could be demonstrated for both Nmu-pdg I and II when reactions were carried out in the presence of sodium borohydride. These data indicate the involvement of an amino group in the catalytic reaction mechanism of both enzymes.

L8 ANSWER 7 OF 24 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000278119 MEDLINE
DOCUMENT NUMBER: 20278119
TITLE: Construction and characterization of Haemophilus ducreyi lipooligosaccharide (LOS) mutants defective in expression of heptosyltransferase III and beta1,4-glucosyltransferase: identification of LOS glycoforms containing lactosamine repeats.
AUTHOR: Filiatrault M J; Gibson B W; Schilling B; Sun S; Munson R S Jr; Campagnari A A
CORPORATE SOURCE: Department of Microbiology, University at Buffalo, Buffalo, New York 14214, USA.
CONTRACT NUMBER: RO1 AI30006 (NIAID)
RO1 AI38444 (NIAID)
RO1 AI31254 (NIAID)
+
SOURCE: INFECTION AND IMMUNITY, (2000 Jun) 68 (6) 3352-61. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-AF215936
ENTRY MONTH: 200009
ENTRY WEEK: 20000901
AB To begin to understand the role of the lipooligosaccharide (LOS) molecule in chancroid infections, we constructed mutants defective in expression of glycosyltransferase genes. Pyocin lysis and immunoscreening was used to identify a LOS mutant of Haemophilus

ducreyi 35000. This mutant, HD35000R, produced a LOS molecule that lacked the monoclonal antibody 3F11 epitope and migrated with an increased mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**). Structural studies indicated that the principal LOS glycoform contains lipid A, Kdo, and two of the three core heptose residues. HD35000R was transformed with a plasmid library of H. ducreyi 35000 **DNA**, and a clone producing the wild-type LOS was identified. Sequence analysis of the plasmid insert revealed one open reading frame (ORF) that encodes a **protein** with homology to the WaaQ (heptosyltransferase III) of Escherichia coli. A second ORF had homology to the LgtF (glucosyltransferase) of **Neisseria** meningitidis. Individual isogenic mutants lacking expression of the putative H. ducreyi heptosyltransferase III, the putative glucosyltransferase, and both glycosyltransferases were constructed and characterized. Each mutant was complemented with the representative wild-type genes in trans to restore expression of parental LOS and confirm the function of each enzyme. Matrix-assisted laser desorption ionization mass spectrometry and **SDS-PAGE** analysis identified several unique LOS glycoforms containing di-, tri-, and poly-N-acetyllactosamine repeats added to the terminal region of the main LOS branch synthesized by the heptosyltransferase III mutant. These novel H. ducreyi mutants provide important tools for studying the regulation of LOS assembly and biosynthesis.

L8 ANSWER 8 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:202759 BIOSIS
DOCUMENT NUMBER: PREV200000202759
TITLE: Cloning and characterization of the lipooligosaccharide galactosyltransferase II gene of Haemophilus ducreyi.
AUTHOR(S): Sun, Shuhua; Schilling, Birgit; Tarantino, Laurie; Tullius, Michael V.; Gibson, Bradford W.; Munson, Robert S., Jr. (1)
CORPORATE SOURCE: (1) Children's Research Institute, 700 Children's Dr., Room W402, Columbus, OH, 43205 USA
SOURCE: Journal of Bacteriology, (April, 2000) Vol. 182, No. 8, pp. 2292-2298.
ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Haemophilus ducreyi is the etiologic agent of chancroid, a genital ulcer disease. The lipooligosaccharide (LOS) is considered to be a major virulence determinant and has been implicated in the adherence of H. ducreyi to keratinocytes. Strain A77, an isolate from the Paris collection, is serum sensitive, poorly adherent to fibroblasts, and deficient in microcolony formation. Structural

analysis indicates that the LOS of strain A77 lacks the galactose residue found in the N-acetyllactosamine portion of the strain 35000HP LOS as well as the sialic acid substitution. From an H. ducreyi 35000HP genomic **DNA** library, a clone complementing the defect in A77 was identified by immunologic screening with monoclonal antibody (MAb) 3F11, a MAb which recognizes the N-acetyllactosamine portion of strain 35000HP LOS. The clone contained a 4-kb insert that was sequenced. One open reading frame which encodes a **protein** with a molecular weight of 33,400 was identified. This **protein** has homology to glycosyltransferases of Haemophilus influenzae, Haemophilus somnus, **Neisseria** species, and Pasteurella haemolytica. The putative H. ducreyi glycosyltransferase gene was insertionally inactivated, and an isogenic mutant of strain 35000HP was constructed. The most complex LOS glycoform produced by the mutant has a mobility on sodium dodecyl sulfate-polyacrylamide gel identical to that of the LOS of strain A77 and lacks the 3F11-binding epitope. Structural studies confirm that the most complex glycoform of the LOS isolated from the mutant lacks the galactose residue found in the N-acetyllactosamine portion of the strain 35000HP LOS. Although previously published data suggested that the serum-sensitive phenotype of A77 was due to the LOS mutation, we observed that the complemented A77 strain retained its serum-sensitive phenotype and that the galactosyltransferase mutant retained its serum-resistant phenotype. Thus, the serum sensitivity of strain A77 cannot be attributed to the galactosyltransferase mutation in strain A77.

L8 ANSWER 9 OF 24 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000005611 MEDLINE
DOCUMENT NUMBER: 20005611
TITLE: Neisseria gonorrhoeae bacterioferritin: structural heterogeneity, involvement in iron storage and protection against oxidative stress.
AUTHOR: Chen C Y; Morse S A
CORPORATE SOURCE: Division of AIDS, Sexually Transmitted Diseases and Tuberculosis Laboratory Research, National Centers for Infectious Disease, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.. cyc1@cdc.gov
SOURCE: MICROBIOLOGY, (1999 Oct) 145 (Pt 10) 2967-75. Journal code: BXW. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U76633; GENBANK-U76634
ENTRY MONTH: 200004
ENTRY WEEK: 20000403
AB The iron-storage **protein** bacterioferritin (Bfr) from **Neisseria** gonorrhoeae strain F62 was identified in cell-free

extracts and subsequently purified by column chromatography. Gonococcal Bfr had an estimated molecular mass of 400 kDa by gel filtration; however, analysis by **SDS-PAGE** revealed that it was composed of 18 kDa (BfrA) and 22 kDa (BfrB) subunits. **DNA** encoding BfrB was amplified by PCR using degenerate primers derived from the N-terminal amino acid sequence of BfrB and from a C-terminal amino acid sequence of Escherichia coli Bfr. The **DNA** sequence of bfrA was subsequently obtained by genome walking using single-specific-primer PCR. The two Bfr genes were located in tandem with an intervening gap of 27 bp. A potential Fur-binding sequence (12 of 19 bp identical to the consensus neisserial fur sequence) was located within the 5' flanking region of bfrA in front of a putative -35 hexamer. The homology between the **DNA** sequences of bfrA and bfrB was 55.7%; the deduced amino acid sequences of BfrA (154 residues) and BfrB (157 residues) showed 39.7% identity, and showed 41.3% and 56.1% identity, respectively, to E. coli Bfr. Expression of recombinant BfrA and BfrB in E. coli strain DH5alpha was detected on Western blots probed with polyclonal anti-E. coli Bfr antiserum. Most Bfrs are homopolymers with identical subunits; however, the evidence presented here suggests that gonococcal Bfr was composed of two similar but not identical subunits, both of which appear to be required for the formation of a functional Bfr. A Bfr-deficient mutant was constructed by inserting the omega fragment into the BfrB gene. The growth of the BfrB-deficient mutant in complex medium was reduced under iron-limited conditions. The BfrB-deficient mutant was also more sensitive to killing by H2O2 and paraquat than the isogenic parent strain. These results demonstrate that gonococcal Bfr plays an important role in iron storage and protection from iron-mediated oxidative stress.

L8 ANSWER 10 OF 24 TOXLINE
ACCESSION NUMBER: 1999:48657 TOXLINE
DOCUMENT NUMBER: CRISP-99-AI43568-01
TITLE: **DNA** SEQUENCE OF STAPHYLOCOCCUS AUREUS 8325 GENOME.
AUTHOR: IANDOLO J J
CORPORATE SOURCE: OKLAHOMA UNIV HLTH SCIS CTR, PO BOX 26901, OKLAMHOMA CITY, OK 73190
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES.
CONTRACT NUMBER: 1R01AI43568-01
SOURCE: (1998). Crisp Data Base National Institutes Of Health. Award Type: G = Grant
PUB. COUNTRY: United States
DOCUMENT TYPE: (RESEARCH)
FILE SEGMENT: CRISP
LANGUAGE: English

ENTRY MONTH: 199904

AB RPROJ/CRISP In 1995, Fleischman et al. (40) determined the **nucleotide** sequence of Haemophilus influenzae Rd without prior restriction site mapping and cosmid cloning. This was a landmark event, signaling that automated **DNA** sequencing and analysis have matured into robust, highly efficient technologies promising to revolutionize the biological sciences. An important breakthrough that will greatly facilitate closure and proof- reading of the sequence which was reported by Dr. Mike Hunkapiller from ABI and Dr. Roe at the recent September Hilton Head **DNA** Sequencing conference was the results of experiments in which we successfully obtained sequence data in excess of 450 bases directly from several bacterial genomes. The genomic sequence of several other procaryotes have or will be completed shortly, including our own genomic sequencing of the important human pathogens **Neisseria** gonorrhoeae and Streptococcus pyogenes. Collectively, these studies will considerably enhance our understanding of procaryotic molecular biological processes, and provide new avenues for gene discovery and comparative genetics. From a practical standpoint, procaryotic genome sequencing will enhance our ability to understand processes occurring during the pathogenesis of infectious disease. These studies should provide new approaches for drug discovery, a necessity of increasing importance as microbial antibiotic resistance threatens our ability to treat bacterial infections. In this application, we propose to determine the **nucleotide** sequence of the genome of Staphylococcus aureus. This organism is a potent pathogen widely found in the human and animal environment. It is capable of producing upwards of 34 different extracellular **proteins** most of which have been shown to play a role in the pathogenicity of the organism or to enhance virulence. Although the incidence of infection in the general population is not well documented, it is generally accepted that S. aureus accounts for up to one-third of all nosocomial bacteremia. Other than the details of abscess formation, very little is actually of the pathogenesis of staphylococcal disease. Understanding the circuits (agr, sar and probably others) which control the expression of virulence related genes is paramount to understanding the genetic response of the organism to host generated signals. We propose that acquisition of the sequence of the genome of the Staphylococcus aureus strain 8325 will greatly facilitate understanding the mechanism of disease produced by this organism and closely related species. Therefore, as a single specific aim, we will sequence and annotate the 2.8 Mb genome of this important pathogen.

L8 ANSWER 11 OF 24 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998136610 MEDLINE
DOCUMENT NUMBER: 98136610
TITLE: Molecular, genetic, and functional analysis of

homozygous C8 beta-chain deficiency in two siblings.
AUTHOR: Kotnik V; Luznik-Bufon T; Schneider P M; Kirschfink M
CORPORATE SOURCE: Institute of Microbiology and Immunology, University of Ljubljana, Slovenia.. vkotnik@ibmi.mf.uni-lj.si
SOURCE: IMMUNOPHARMACOLOGY, (1997 Dec) 38 (1-2) 215-21.
Journal code: GY3. ISSN: 0162-3109.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY WEEK: 19980604
AB C8 deficiency is associated with an increased susceptibility to neisserial infections. We present a case of an 11 year old boy who suffered from infection with **Neisseria** meningitidis. Medical history of the patient and his family (n = 5) did not indicate any previous immunodeficiency symptoms. Results from the analysis of phagocyte and lymphocyte functions were within the normal range. No hemolytic activities of the classical (CH50) and the alternative (APH50) pathways of complement were measurable, and SC5b-9 **protein** complexes could not be detected in the patient's plasma. Further analysis by highly sensitive ELISA and functional assays revealed a complete deficiency of C8. Upon the reconstitution with purified C8 total hemolytic activity could be restored. **SDS-PAGE** and Western blot analysis established a deficiency of the C8 beta chain. Genetic analysis at the genomic **DNA** level demonstrated the common C-T mutation in exon 9 of the C8B gene. Family analysis presented the older sister with non-detectable function of C8 in serum, both parents with about half-normal C8 titres, and the younger sister with normal C8 function. The parents and both sisters were asymptomatic, although the older of the sisters presented with the same complete C8 beta-chain deficiency as the patient described. In conclusion: the common C-T mutation in the C8B genes is the genetic basis of C8 beta-chain deficiency in two members of this Bosnian family.

L8 ANSWER 12 OF 24 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998353647 MEDLINE
DOCUMENT NUMBER: 98353647
TITLE: The aroQ and pheA domains of the bifunctional P-protein from Xanthomonas campestris in a context of genomic comparison.
AUTHOR: Gu W; Williams D S; Aldrich H C; Xie G; Gabriel D W; Jensen R A
CORPORATE SOURCE: Department of Microbiology and Cell Science, University of Florida, Gainesville, USA.
SOURCE: MICROBIAL AND COMPARATIVE GENOMICS, (1997) 2 (2) 141-58.
Journal code: C5D. ISSN: 1090-6592.
Searcher : Shears 308-4994

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U64518
ENTRY MONTH: 199810
ENTRY WEEK: 19981004

AB The gene (denoted aroQp.pheA) encoding the bifunctional P-**protein** (chorismate mutase-P/prephenate dehydratase) from Xanthomonas campestris was cloned. aroQp.pheA is essential for L-phenylalanine biosynthesis. **DNA** sequencing of the smallest subclone capable of functional complementation of an Escherichia coli phenylalanine auxotroph revealed a putative open reading frame (ORF) of 1200 bp that would encode a 43,438-Da **protein**. AroQp.PheA exhibited 51% amino acid identity with a Pseudomonas stutzeri homologoue and greater than 30% identities with AroQp.PheA **proteins** from Haemophilus influenzae, **Neisseria** gonorrhoeae, and a number of enteric bacteria. AroQp.PheA from X. campestris, when expressed in E. coli, possesses a 40-residue amino-terminal extension that is lysine-rich and that is absent in all of the AroQp.PheA homologues known at present. About 95% of AroQp.PheA was particulate and readily sedimented by low-speed centrifugation. Soluble preparations of cloned AroQp.PheA exhibited a native molecular mass of 81,000 Da, indicating that the active enzyme species is a homodimer. These preparations were unstable after purification of about 40-fold, even in the presence of glycerol, which was an effective protectant before fractionation. When AroQp.PheA was overproduced by a T7 translation vector, unusual inclusion bodies having a macromolecular structure consisting of **protein** fibrils were observed by electron microscopy. Insoluble **protein** collected at low-speed centrifugation possessed high catalytic activity. The single band obtained via **SDS-PAGE** was used to confirm the translational start via N-terminal amino acid sequencing. A perspective on the evolutionary relationships of monofunctional AroQ and PheA **proteins** and the AroQp.PheA family of **proteins** is presented. A serC gene located immediately upstream of X. campestris aroQp.pheA appears to reflect a conserved gene organization, and both may belong to a single transcriptional unit.

L8 ANSWER 13 OF 24 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97039866 MEDLINE
DOCUMENT NUMBER: 97039866
TITLE: Proline iminopeptidase gene from Xanthomonas campestris pv. citri.
AUTHOR: Alonso J; Garcia J L
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Consejo Superior de Investigaciones Cientificas, Velazquez, Madrid, Spain.

SOURCE: MICROBIOLOGY, (1996 Oct) 142 (Pt 10) 2951-7.
Journal code: BXW. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z54150
ENTRY MONTH: 199703

AB The pip gene coding for the proline iminopeptidase (Pip) of Xanthomonas campestris pv. citri was cloned in an Escherichia coli leuB strain using a selective medium containing the dipeptide D-Ala-L-Leu as the sole source of L-leucine. **Nucleotide** sequencing of this gene revealed a 939 bp open reading frame encoding a 312 amino acid **protein** (35 126 Da). The deduced amino acid sequence showed 47% identity with the Pip from **Neisseria** gonorrhoeae. A lacZ-pip fusion gene was overexpressed in E. coli under the control of the Plac promoter. The Pip of X. campestris hydrolysed L-prolyl-p-nitroanilide with the highest efficiency, but was also able to hydrolyse L-alanyl-p-nitroanilide and D-alanyl-p-nitroanilide. The molecular mass of Pip was found to be 35 kDa by **SDS-PAGE** and 120 kDa by gel filtration, suggesting that the active enzyme is a multimer.

L8 ANSWER 14 OF 24 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 96258475 EMBASE
DOCUMENT NUMBER: 1996258475

TITLE: [Molecular epidemiology of meningitis due to **Neisseria** meningitidis in Mali: Isolation of a new variant (P1.y) of the class 1 **protein**].
EPIDEMIOLOGIE MOLECULAIRE DE LA MENINGITE A MENINGOCOQUE AU MALI: ISOLEMENT D'UN NOUVEAU VARIANT (P1.Y) DE LA PROTEINE DE CLASSE 1.

AUTHOR: Koumare B.; Achtman M.; Bougoudogo F.; Cisse M.; Wang J.F.
CORPORATE SOURCE: Inst. Nat. de Rech. en Sante Publ., Service de Bacteriologie-Virologie, BP 1771,Bamako, Mali

SOURCE: Bulletin of the World Health Organization, (1996) 74/4 (375-379).
ISSN: 0042-9686 CODEN: BWHOA6
COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
008 Neurology and Neurosurgery
017 Public Health, Social Medicine and Epidemiology
LANGUAGE: French
SUMMARY LANGUAGE: English; French

AB The study deals with 570 strains of Neisseriaceae isolated between 1989 and 1994 in Mali: 396 of the strains were isolated from samples of cerebrospinal fluid and 174 from the throat. Serogroup C accounted for 55% of all strains. Antigenic structure was determined by ELISA, **SDS-PAGE** and transfer to nitrocellulose membrane for immunoblotting with monoclonal antibodies produced at the Max Planck Institute for Molecular Genetics. For serogroup A, the class 1 protein types found were P1.7 for strains isolated prior to 1994 and P1.9 for strains isolated in 1994. P1.7 is specific to clone IV-1 and P1.9 to clone III-1, which was responsible for the 1994 epidemic. All strains of serogroup C isolated from fluid CSF and most strains isolated from the throat exhibit a new type of class 1 protein which the authors have designated P1.y. P1.y is characteristic of Malian strains of serogroup C; it is rare or absent in strains from other countries (Burkina Faso, Ghana, Italy, USA). The **nucleotide** sequence of the gene expressing P1.y and the corresponding amino acid sequence were determined at the National Institute for Biological Standards and Control, England.

L8 ANSWER 15 OF 24 MEDLINE
ACCESSION NUMBER: 96423180 MEDLINE
DOCUMENT NUMBER: 96423180
TITLE: Identification and characterization of genes required for post-translational modification of Campylobacter coli VC167 flagellin.
AUTHOR: Guerry P; Doig P; Alm R A; Burr D H; Kinsella N; Trust T J
CORPORATE SOURCE: Enteric Diseases Program, Naval Medical Research Institute, Bethesda, Maryland 20889, USA.. guerry_p@mail2.nmri.nnmc.navy.mil
SOURCE: MOLECULAR MICROBIOLOGY, (1996 Jan) 19 (2) 369-78. Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U25992
ENTRY MONTH: 199702

AB Two genes have been identified in Campylobacter coli VC167 which are required for the biosynthesis of post-translational modifications on flagellin **proteins**. The ptmA gene encodes a **protein** of predicted M(r) 28,486 which shows significant homology to a family of alcohol dehydrogenases from a variety of bacteria. The ptmB gene encodes a **protein** of predicted M(r) 26,598 with significant homology to CMP-N-acetylneuraminic acid synthetase enzymes involved in sialic acid capsular biosynthesis in **Neisseria** meninigitidis and Escherichia coli K1. Site-specific mutation of either ptmA or ptmB caused loss of

reactivity with antisera specific to the post-translational modifications and a change in the isoelectric focusing fingerprints relative to the parent strains. Mutation of ptmB, but not of ptmA, caused a change in apparent M(r) of the flagellin subunit in **SDS-PAGE** gels. The ptmA and ptmB genes are present in other strains of Campylobacter. In a rabbit model the ptmA mutant showed a reduced ability to elicit protection against subsequent challenge with heterologous strains of the same Lior serotype compared to the parental wild-type strain. This suggests that the surface-exposed post-translational modifications may play a significant role in the protective immune response.

L8 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:267378 BIOSIS
DOCUMENT NUMBER: PREV199698823507
TITLE: Tandem repeats of the tetramer 5'-CAAT-3' present in lic2A are required for phase variation but not lipopolysaccharide biosynthesis in Haemophilus influenzae.
AUTHOR(S): High, Nicola J. (1); Jennings, Michael P.; Moxon, E. Richard
CORPORATE SOURCE: (1) 1.800, Stopford Building, Sch. Biol. Sci., Univ. Manchester, Manchester M13 9PT UK
SOURCE: Molecular Microbiology, (1996) Vol. 20, No. 1, pp. 165-174.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A novel lipopolysaccharide (LPS) biosynthesis gene, lic2B, which is required for the biosynthesis of a phase-variable LPS structure expressed by Haemophilus influenzae RM7004 is described. The product of this gene is homologous to Lic2A and the recently described LPS biosynthetic enzymes, LgtB from **Neisseria** gonorrhoea and LgtE from **Neisseria** meningitidis, and LpsA from Pasteurella haemolytica. Of this family of enzymes only Lic2A contains the repetitive tetrapeptide motif (SINQ)-n encoded by multiple tandem repeats of 5'-CAAT-3'. This observation suggested that (SINQ)-n might not be a prerequisite for the catalytic activity of this **protein**. To address this hypothesis, we deleted the 5'-CAAT-3' repeats from lic2A so that the **protein** encoded by the modified gene was analogous to Lic2B. This mutation had no apparent effect on the overall apparent molecular weight of LPS as judged by Tricine-**SDS-PAGE** and did not affect ability to react with monoclonal antibody 4C4. It was therefore concluded that (SINQ)-n is not a prerequisite for the enzymatic function of Lic2A and that the 5'-CAAT-3' repeats in lic2A function solely as a mechanism for generating phase variation. This observation suggested that wide variation in the number of 5'-CAAT-3' repeats might be tolerated in lic2A, and this was

confirmed by surveying the number of 5'-CAAT-3' repeats in a range of different H. influenzae strains. The predicted secondary structure of (SINQ)-n indicates that it forms a highly flexible random coiled structure, which is unlikely to impede formation of the domains that may be required for catalytic activity. This characteristic is also a feature of repetitive tetrapeptides encoded by other tetrameric repeats located within coding sequences present on the chromosome of H. influenzae Rd.

L8 ANSWER 17 OF 24 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 95198536 MEDLINE
DOCUMENT NUMBER: 95198536
TITLE: Lipooligosaccharide biosynthesis in Neisseria gonorrhoeae: cloning, identification and characterization of the alpha 1,5 heptosyltransferase I gene (rfaC) [published erratum appears in Mol Microbiol 1995 Apr;16(1):169].
AUTHOR: Zhou D; Lee N G; Apicella M A
CORPORATE SOURCE: Department of Microbiology, University of Iowa, Iowa City 52242..
CONTRACT NUMBER: AI18384 (NIAID)
AI24616 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1994 Nov) 14 (4) 609-18.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U10385
ENTRY MONTH: 199506
AB The identical partial deep-core structure of Hep alpha 1-3Hep alpha 1-5KDO in Salmonella typhimurium LT2 LPS and **Neisseria** gonorrhoeae LOS enabled us to isolate a **DNA** fragment from N. gonorrhoeae that was able to complement the alpha 1,5 LOS heptosyltransferase defect in the S. typhimurium rfaC630 (SA1377) mutant. **SDS-PAGE** analysis confirmed the production of wild-type LPS in the transformant. Subcloning revealed that complementation was due to a 1.2 kb fragment. Sequence analysis revealed a complete open reading frame capable of encoding a 36-37 kDa **peptide**. In vitro transcription-translation analysis of the 1.2 kb clone confirmed that a 37 kDa **protein** was encoded by this **DNA** fragment. The **DNA** sequence-deduced **protein** had 36% identity and 58% similarity to S. typhimurium heptosyltransferase I (RfaC). Primer extension analysis indicated that transcription of the cloned gene in N. gonorrhoeae strain 1291 begins 144 bp upstream of the start codon at a G **nucleotide**. An isogenic mutant of N. gonorrhoeae strain 1291 with an m-Tn3 insertion inside the coding sequence expressed a single truncated LOS with a similar molecular

mass to S. typhimurium rfaC LPS. We conclude that the 1.2 kb fragment encodes the alpha 1,5 LOS heptosyltransferase I (RfaC) in N. gonorrhoeae. Our studies also provide further evidence that the third KDO residue in S. typhimurium LPS is added after the core synthesis is completed.

L8 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:176611 BIOSIS
DOCUMENT NUMBER: PREV199497189611
TITLE: Genetic diversity of the iron-binding **protein** (Fbp) gene of the pathogenic and commensal **Neisseria**.
AUTHOR(S): Genco, Caroline Attardo (1); Berish, Sally A.; Chen, Cheng-Yen; Morse, Stephen; Trees, David L.
CORPORATE SOURCE: (1) Dep. Microbiol. Immunol., Morehouse Sch. Med., Atlanta, GA 30310 USA
SOURCE: FEMS Microbiology Letters, (1994) Vol. 116, No. 2, pp. 123-129.
ISSN: 0378-1097.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The pathogenic **Neisseria** and most commensal **Neisseria** species produce an iron-binding **protein** (Fbp) when grown under iron-limited conditions. In the current study, we confirmed the presence of Fbp, as well as **DNA** sequences homologous to the gonococcal fbp, in strains of V. gonorrhoeae, N. meningitidis, N. cinerea, N. lactamica, N. subflava, N. kochii and N. polysaccharea, The fbp genes from these strains were amplified by the polymerase chain reaction, digested with StuI or RsaI, and the restriction patterns examined. The patterns for the gonococcal and meningococcal fbp were virtually identical; however, variations were observed in the fbp sequences of the commensal **Neisseria** species. N. flauescens, N. mucosa, N. sicca, N. ovis and Branhamella catarrhalis, did not produce Fbp as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and reactivity with an Fbp specific monoclonal antibody, nor did they hybridize to an fbp-specific **DNA** probe.

L8 ANSWER 19 OF 24 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 95058178 MEDLINE
DOCUMENT NUMBER: 95058178
TITLE: Expression of meningococcal epitopes in LamB of Escherichia coli and the stimulation of serosubtype-specific antibody responses.
AUTHOR: McCarvil J; McKenna A J; Grief C; Hoy C S; Sesardic D; Maiden M C; Feavers I M
CORPORATE SOURCE: Division of Bacteriology, National Institute for Biological Standards and Control, South Mimms, Hertfordshire, UK..
Searcher : Shears 308-4994

SOURCE: MOLECULAR MICROBIOLOGY, (1993 Oct) 10 (1) 203-13.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502

AB The class 1 outer membrane **protein** (OMP), a major variable surface antigen of **Neisseria** meningitidis, is a component of novel meningococcal vaccines currently in field trials. Serological variants of the **protein** are also used to serosubtype meningococci. Most of the amino acid changes that give rise to antigenic variants of the **protein** occur in two variable regions (VR1 and VR2) that are thought to form loops on the cell surface. The polymerase chain reaction (PCR) was used to amplify the **nucleotide** sequences encoding VR1 and VR2 from the chromosomal **DNA** of N. meningitidis strain M1080. These were cloned in frame into the lamB gene of the Escherichia coli expression vector pAJC264. Whole-cell enzyme-linked immunosorbent assays (ELISAs), using monoclonal antibodies, and **SDS-PAGE** confirmed that, upon induction, strains of E. coli carrying these constructs expressed hybrid LamB **proteins** containing the N. meningitidis surface loops. These strains were used to immunize rabbits and the resultant polyclonal antisera reacted specifically with the class 1 OMP of reference strain M1080 (P1.7). Immunogold labelling of meningococcal cells and whole-cell dot-blot analyses with these antisera showed that the variable epitopes were exposed on the cell surface and confirmed that this approach could be used to obtain serosubtype-specific antisera. The binding profiles of the antisera were determined from their reactions with overlapping synthetic **peptides** and their reactivity compared with that of relevant serosubtype-specific monoclonal antibodies. This approach was used successfully to raise antisera against two other class 1 OMP VR2s. A fourth antiserum raised against a VR2, including the P1.1 epitope, was not subtype specific.

L8 ANSWER 20 OF 24 EMBASE

ACCESSION NUMBER: 93273095 EMBASE
DOCUMENT NUMBER: 1993273095
TITLE: Neisseria gonorrhoeae strain MS11 harbouring a mutation in gene aroA is attenuated and immunogenic.
AUTHOR: Chamberlain L.M.; Strugnell R.; Dougan G.; Hormaeche C.E.; Demarco de Hormaeche R.
CORPORATE SOURCE: Department of Pathology, Tennis Court Road,Cambridge CB2 1QP, United Kingdom
SOURCE: Microbial Pathogenesis, (1993) 15/1 (51-63).
ISSN: 0882-4010 CODEN: MIPAEV
COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
013 Dermatology and Venereology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB An aroA mutant of gonococcal strain MS11 was constructed (JKD298) and compared with the wild-type (JKD288). The requirement of JKD298 for aromatic compounds, typical of an aroA mutant, was demonstrated using defined media. Other than the expected auxotrophy, no further differences could be demonstrated between the parent and the aroA mutant. **SDS-PAGE** analysis of **protein** and lipopolysaccharide (LPS) profiles showed no differences between the strains. Bactericidal assays using human and guinea-pig normal sera showed that both strains were serum sensitive and were similarly converted to serum resistance by in vitro sialylation using CMP-NANA. Infectivity experiments in guinea-pig subcutaneous chambers showed considerably reduced virulence of the aroA JKD298, which could only infect chambers at very high doses. Established infections by either strain elicited a strong PMN response in which similar proportions of each strain were seen intracellularly. Infections by JKD298 provoked a strong antibody response as detected by ELISA using whole sonicated gonococci. This is the first demonstration of attenuation of **Neisseria** gonorrhoeae by introduction of a defined mutation in a metabolic gene.

L8 ANSWER 21 OF 24 TOXLIT
ACCESSION NUMBER: 1993:59032 TOXLIT
DOCUMENT NUMBER: CA-118-226653G
TITLE: Identification of meningococcal serosubtypes by polymerase chain reaction.
AUTHOR: Maiden MC J; Bygraves JA; McCarvil J; Feavers IM
CORPORATE SOURCE: Nal. Inst. Biol. Stand. Control, Hertsfordshire
SOURCE: J. Clin. Microbiol, (1992). Vol. 30, No. 11, pp. 2835-41.
CODEN: JCMIDW. ISSN. 0095-1137.
PUB. COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 118:226653
ENTRY MONTH: 199307

AB The polymerase chain reaction was used as the basis of a novel typing method for **Neisseria** meningitidis. Southern hybridization expts. demonstrated that it was possible to identify genes encoding different serol. variants of the meningococcal class 1 outer membrane **protein** by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14

defined variable regions was prepd. in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane **protein** genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one expt. This technique was evaluated with a set of serol. ref. strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of **SDS-polyacrylamide** gel **electrophoresis** or serol. anal. An addnl. advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the **DNA** amplified from the original sample could be used to det. the **nucleotide** sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clin. samples even when culture is not possible because of antibiotic treatment of an acute case.

L8 ANSWER 22 OF 24 MEDLINE
ACCESSION NUMBER: 91346008 MEDLINE
DOCUMENT NUMBER: 91346008
TITLE: Characterization of a soluble ferric reductase from Neisseria gonorrhoeae.
AUTHOR: Le Faou A E; Morse S A
CORPORATE SOURCE: Division of Sexually Transmitted Diseases Laboratory Research, Centers for Disease Control, Atlanta, Georgia 30333.
SOURCE: BIOLOGY OF METALS, (1991) 4 (2) 126-31. Journal code: AU2. ISSN: 0933-5854.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112

AB An NADH-dependent ferric reductase was identified in extracts of **Neisseria** gonorrhoeae. Enzyme activity was measured in an assay using ferrozine as the ferrous iron acceptor. Ferric reductase activity was enhanced by Mg2+ and flavine **nucleotides**. The enzyme reduced both citrate- and diphosphate-bound ferric iron as well as ferric hydroxide (Imferon). However, no activity was observed with either 30%-iron-saturated transferrin or with the gonococcal iron-binding **protein**, Fbp. The ferric reductase was found primarily within the cytoplasmic cell fraction. The soluble ferric reductase was purified 110-fold by ammonium sulfate precipitation, gel and anion-exchange chromatography. Results

obtained following gel chromatography and **SDS/polyacrylamide** gel **electrophoresis** suggested that the enzyme had a molecular mass of about 25 kDa.

L8 ANSWER 23 OF 24 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 90355837 MEDLINE
DOCUMENT NUMBER: 90355837
TITLE: Stable expression of meningococcal class 1 protein in an antigenically reactive form in outer membranes of Escherichia coli.
AUTHOR: White D A; Barlow A K; Clarke I N; Heckels J E
CORPORATE SOURCE: Department of Microbiology, University of Southampton Medical School, Southampton General Hospital, UK..
SOURCE: MOLECULAR MICROBIOLOGY, (1990 May) 4 (5) 769-76. Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199011

AB The entire gene encoding the class 1 outer membrane **protein** of **Neisseria** meningitidis is located on a 2.2kb fragment, obtained on digestion of chromosomal **DNA** with Xbal. This Xbal fragment from strain MC50 (subtype P1-16), which had previously been cloned in bacteriophage M13, has been transferred to the plasmid vector pMTL20. The resulting plasmid (pPORA100) was propagated in Escherichia coli (JM109) and cell lysates were subjected to **SDS-PAGE**. Western blotting with anti-class 1 **protein** antibodies revealed constitutive expression of a **protein** of 41 kD, corresponding to the class 1 **protein** of the parent meningococcal strain, which was absent in the E. coli control. Fractionation of E. coli cells carrying the recombinant plasmid revealed that the **protein** was exclusively located in the outer membrane, and N-terminal amino acid analysis of the expressed **protein** revealed that normal processing of the signal **peptide** had occurred. Immuno-gold electron microscopy showed that the protective epitope recognized by a P1-16 subtype-specific monoclonal antibody was exposed in an antigenically reactive form on the surface of E. coli cells carrying plasmid pPORA100. In contrast, expression in E. coli of a second plasmid (pPORA104) lacking the coding sequence for the first 15 amino acids of the signal **peptide** resulted in accumulation of recombinant class 1 **protein** only in the cytoplasm of the cells. Thus the presence of the meningococcal signal sequence ensures expression of this meningococcal porin **protein** in an antigenically native conformation in outer membranes of E. coli, while its absence results in expression of a soluble **protein**. (ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 24 OF 24 MEDLINE DUPLICATE 10

ACCESSION NUMBER: 86252506 MEDLINE

DOCUMENT NUMBER: 86252506

TITLE: An unusual Neisseria isolated from conjunctival cultures in rural Egypt.

AUTHOR: Mazloum H; Totten P A; Brooks G F; Dawson C R; Falkow S; James J F; Knapp J S; Koomey J M; Lammel C J; Peters D; et al

CONTRACT NUMBER: AI-15642 (NIAID)
AI-21912 (NIAID)
EY-00427 (NEI)
+

SOURCE: JOURNAL OF INFECTIOUS DISEASES, (1986 Aug) 154 (2) 212-24.
Journal code: IH3. ISSN: 0022-1899.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 198610

AB Seven isolates of an unusual **Neisseria** sp. were obtained from eye cultures of children in two rural Egyptian villages. These **Neisseria** utilized only glucose, they exhibited a positive reaction when tested with antisera to crude antigen from **Neisseria** meningitidis and N. gonorrhoeae, and they did not react with the fluorescent antibody tests for N. gonorrhoeae or with the monoclonal antibodies used to serotype gonococci. The Egyptian isolates had colony morphology more typical of meningococci than gonococci and showed opaque and transparent colony variants. On **SDS-PAGE**, the major outer-membrane **proteins** had different patterns than those noted for comparable **proteins** of meningococci and gonococci; heat-modifiable outer-membrane **proteins** were present. Four of the six isolates examined had cryptic plasmids of 2.8 megadaltons, which were slightly larger than the cryptic plasmid of N. gonorrhoeae. These plasmids were homologous to the gonococcal cryptic plasmid, but had different restriction enzyme fragment patterns. The **DNA** from the Egyptian isolates, like **DNA** from N. meningitidis but unlike **DNA** from N. gonorrhoeae, could be cut with the restriction enzyme HaeIII. The frequency of transformation into a temperature-sensitive mutant of N. gonorrhoeae was 0.2 for the Egyptian isolates and 0.1 for N. meningitidis, a frequency that was 5-10-fold lower than that for the N. gonorrhoeae control isolates. Whole-cell **DNA** from the Egyptian isolates showed 68%-73% homology with N. gonorrhoeae and 57%-63% with N. meningitidis. On the basis of our observations, the Egyptian isolates are distinct from N. meningitidis and may represent a variant of N. gonorrhoeae. We suggest that the isolates be called **Neisseria** gonorrhoeae ssp. kochii.

Searcher : Shears 308-4994

FILE 'CAPLUS' ENTERED AT 09:59:45 ON 22 MAR 2001
L9 6 S L5 AND ((NA OR SODIUM)(W)DODECYL(1W)(PAGE OR (POLYACRYL? OR POLY ACRYL?)(1W)ELECTROPHOR?)
L10 5 S L9 NOT L6

L10 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:371105 CAPLUS
DOCUMENT NUMBER: 133:218385
TITLE: Sequence variation in the porA gene of a clone of Neisseria meningitidis during epidemic spread
AUTHOR(S): Jelfs, J.; Munro, R.; Wedege, E.; Caugant, D. A.
CORPORATE SOURCE: WHO Collaborating Centre for Reference and Research on Meningococci, Department of Bacteriology, National Institute of Public Health, Oslo, N-0403, Norway
SOURCE: Clin. Diagn. Lab. Immunol. (2000), 7(3), 390-395
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The ET-15 clone within the electrophoretic type (ET)-37 complex of Neisseria meningitidis was 1st detected in Canada in 1986 and was since assocd. with outbreaks of meningococcal disease in many parts of the world. While the majority of the strains of the ET-37 complex are serosubtype P1.5,2, serosubtype detn. of ET-15 strains may often be incomplete, with either only 1 or none of the 2 variable regions (VRs) of the serosubtype PorA outer membrane protein reacting with monoclonal antibodies. **DNA** sequence anal. of the porA gene from ET-15 strains with 1 or both unidentified serosubtype determinants was undertaken to identify the genetic basis of the lack of reaction with the monoclonal antibodies. 14 Different porA alleles were identified among 38 ET-15 strains from various geog. origins. The sequences corresponding to subtypes P1.5a,10d, P1.5,2, P1.5,10d, P1.5a,10k, and P1.5a,10a were identified in 18, 11, 2, 2, and 1 isolate, resp. Of the remaining 4 strains, which all were nonserosubtypeable, 2 had a stop codon within the VR1 and the VR2, resp., while in the other 2 the porA gene was interrupted by the insertion element, IS1301. Of the strains with P1.5,2 sequence, one had a stop codon between the VR1 and VR2, one had a 4-amino-acid deletion outside the VR2, and another showed no expression of PorA on **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**. Our results reveal that numerous genetic events have occurred in the porA gene of the ET-15 clone in the short time of its epidemic spread. The magnitude of microevolutionary mechanisms available in meningococci and the remarkable genetic flexibility of these bacteria need to be considered in relation to PorA vaccine development.
REFERENCE COUNT: 37

REFERENCE(S):

(3) Arhin, F; Can J Microbiol 1998, V44, P56 CAPLUS
(5) Barlow, A; Mol Microbiol 1989, V3, P131 CAPLUS
(10) Feavers, I; Clin Diagn Lab Immunol 1996, V3, P444 CAPLUS
(11) Feavers, I; Mol Microbiol 1992, V6, P489 CAPLUS
(15) Hammerschmidt, S; EMBO J 1996, V15, P192 CAPLUS

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L10 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:722364 CAPLUS
DOCUMENT NUMBER: 126:27472
TITLE: Two glycosyltransferase genes, lgtF and rfaK, constitute the lipooligosaccharide ice (inner core extension) biosynthesis operon of Neisseria meningitidis
AUTHOR(S): Kahler, Charlene M.; Carlson, Russell W.; Rahman, M. Mahbubur; Martin, Larry E.; Stephens, David S.
CORPORATE SOURCE: Deps. Medicine and Microbiol. and Immunology, Emory Univ. School Medicine, Atlanta, GA, T, USA
SOURCE: J. Bacteriol. (1996), 178(23), 6677-6684
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have characterized an operon required for inner-core biosynthesis of the lipooligosaccharide (LOS) of Neisseria meningitidis. Using Tn916 mutagenesis, the authors recently identified the .alpha.-1,2-N-acetylglucosamine (GlcNAc) transferase gene (rfaK), which when inactivated prevents the addn. of GlcNAc and .alpha. chain to the meningococcal LOS inner core. During the study of rfaK, a second open reading frame (lgtF) of 720 bp was found upstream of rfaK. An amino acid sequence homol. search of the GenBank and EMBL databases revealed that the amino terminus of LgtF has significant homol. with a family of .beta.-glycosyltransferases involved in the biosynthesis of polysaccharides and O antigen of lipopolysaccharides. The chromosomal copy of lgtF was mutagenized with a nonpolar antibiotic resistance cassette to minimize potential polar effects on rfaK. Tricine **sodium dodecyl sulfate-polyacrylamide** gel **electrophoresis** and compn. anal. of the LOS from the nonpolar lgtF mutant showed that this strain produced a truncated LOS structure which contained a LOS inner core of GlcNAc1Hep2KDO2 lipid A but without the addn. of lacto-N-neotetraose to HepI or glucose to HepII. These results and the amino acid homol. with .beta.-glycosyltransferases suggest that

lgtF encodes the UDP-glucose:LOS-.beta.-1,4-glucosyltransferase which attaches the first glucose residue to HepI of LOS. Reverse transcriptase PCR and primer extension anal. indicate that both lgtF and rfaK are cotranscribed as a polycistronic message from a promoter upstream of lgtF. This arrangement suggests that completion of the LOS inner core and the initiation of the .alpha. chain addn. are tightly coregulated in N. meningitidis.

L10 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:545679 CAPLUS
DOCUMENT NUMBER: 123:2623
TITLE: Interaction of the **Neisseria** gonorrhoeae PilA **protein** with the pilE promoter involves multiple sites on the **DNA**
AUTHOR(S): Arvidson, Cindy Grove; So, Magdalene
CORPORATE SOURCE: Dep. Molec. Microbiol. Immunol., Oregon Health Sci. Univ., Portland, OR, 97201-3098, USA
SOURCE: J. Bacteriol. (1995), 177(9), 2497-504
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB PilA is the putative **DNA**-binding component of a two-component system that regulates transcription of the pilin expression locus (pilE) of Neisseria gonorrhoeae. Here we report the purifn. of the PilA protein and characterization of its **DNA**-binding activity. PilA was overproduced in Escherichia coli with an isopropyl-.beta.-D-thiogalactopyranoside (IPTG)-inducible expression vector. Cell exts. were prepd. by sonication and fractionated by anion-exchange chromatog., followed by dye affinity chromatog. with Cibacron Blue. Proteins were eluted by using a gradient of KCl, and PilA-contg. fractions were identified by immunoblot anal. with a polyclonal anti-PilA antiserum. Purified PilA was judged to be >90% pure, as detd. by Coomassie blue staining and **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**.
PilA purified in this manner was used to develop a gel retardation assay with a 301-bp fragment contg. the pilE promoter (PpilE) and upstream sequences as a probe. A fragment of similar size contg. the E. coli aroH promoter was used as a neg. control. Competition expts. using a 100- to 1,000-fold excess of unlabeled **DNA** fragments confirmed the specificity of PilA binding to the pilE promoter. To localize the PilA binding site within the 301-bp PpilE fragment, stepwise deletions were generated by PCR and the fragments were examd. in the gel shift assay. The results of these expts. show that there are two regions upstream of PpilE that are required for binding by PilA. Taken together, these data indicate that while PilA binds specifically to the upstream region of the pilE gene, this interaction is complex and likely involves multiple regions of

this **DNA** sequence.

L10 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:291104 CAPLUS
DOCUMENT NUMBER: 120:291104
TITLE: Genetic diversity of the iron-binding **protein** (Fbp) gene of the pathogenic and commensal **Neisseria**
AUTHOR(S): Genco, Caroline Attardo; Berish, Sally A.; Chen, Chengyen; Morse, Stephen; Trees, David L.
CORPORATE SOURCE: Dep. Microbiol. Immunol., Morehouse Sch. Med., Atlanta, GA, 30310, USA
SOURCE: FEMS Microbiol. Lett. (1994), 116(2), 123-9
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The pathogenic **Neisseria** and most commensal **Neisseria** species produce an iron-binding **protein** (Fbp) when grown under iron-limited conditions. In the current study, the authors confirmed the presence of Fbp, as well as **DNA** sequences homologous to the gonococcal fbp, in strains of N. gonorrhoeae, N. meningitidis, N. cinerea, N. lactamica, N. subflava, N. kochii and N. polysaccharea. The fbp genes from these strains were amplified by the polymerase chain reaction, digested with StuI or RsaI, and the restriction patterns examd. The patterns for the gonococcal and meningococcal fbp were virtually identical; however, variations were obsd. in the fbp sequences of the commensal Neisseria species. N. flavescens, N. mucosa, N. sicca, N. ovis and Branhamella catarrhalis, did not produce Fbp as detected by **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis** and reactivity with an Fbp specific monoclonal antibody, nor did they hybridize to an fbp-specific **DNA** probe.

L10 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:161990 CAPLUS
DOCUMENT NUMBER: 118:161990
TITLE: Analysis of the lsi region involved in lipooligosaccharide biosynthesis in Neisseria gonorrhoeae
AUTHOR(S): Petricoin, Emanuel F., III; Danaher, Robert J.; Stein, Daniel C.
CORPORATE SOURCE: Dep. Microbiol., Univ. Maryland, College Park, MD, 20742, USA
SOURCE: J. Bacteriol. (1991), 173(24), 7896-902
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The genetic locus (lsi-1) responsible for the transformation of the

lipooligosaccharide (LOS)-defective N. gonorrhoeae mutant FA5100 to LOS expression was studied by deletion mutagenesis and sequence anal. An open reading frame that was preceded by a leader sequence contg. regions with the potential to form hairpin loops was identified. A perfect .sigma.70 promoter consensus was found upstream from this open reading frame. Promoter function was screened for functionality by using lac fusion cassettes and in vitro transcription-translation anal. A frameshift mutation in the lsi-1 gene was constructed by site-directed mutagenesis and introduced into the chromosome of FA19, the LOS-expressing isogenic parent strain of FA5100. The mutant was characterized by Southern blotting, **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**, and Western blotting (immunoblotting) and found to be phenotypically identical to FA5100.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 10:02:13 ON 22 MAR 2001)

L11 30 S L9
L12 24 S L11 NOT L7
L13 8 DUP REM L12 (16 DUPLICATES REMOVED)

L13 ANSWER 1 OF 8 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001035739 EMBASE
TITLE: Analysis of lipooligosaccharide biosynthesis in the Neisseriaceae.
AUTHOR: Arking D.; Tong Y.; Stein D.C.
CORPORATE SOURCE: D.C. Stein, Dept. of Cell Biology/Molec. Genet., University of Maryland, College Park, MD 20742, United States. DS64@UMAIL.UMD.EDU
SOURCE: Journal of Bacteriology, (2001) 183/3 (934-941). Refs: 51
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Neisserial lipooligosaccharide (LOS) contains three oligosaccharide chains, termed the .alpha., .beta., and .gamma. chains. We used Southern hybridization experiments on **DNA** isolated from various **Neisseria** spp. to determine if strains considered to be nonpathogenic possessed **DNA** sequences homologous with genes involved in the biosynthesis of these oligosaccharide chains. The presence or absence of specific genes was compared to the LOS profiles expressed by each strain, as characterized by their mobilities on **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis** gel and their reactivities with various LOS-specific monoclonal antibodies. A

great deal of heterogeneity was seen with respect to the presence of genes encoding glycosyltransferases in **Neiss ria**. All pathogenic species were found to possess **DNA** sequences homologous with the lgt gene cluster, a group of genes needed for the synthesis of the .alpha. chain. Some of these genes were also found to be present in strains considered to be nonpathogenic, such as **Neisseria** lactamica, N. subflava, and N. sicca. Some nonpathogenic **Neisseria** spp. were able to express high-molecular-mass LOS structures, even though they lacked the **DNA** sequences homologous with rfaF, a gene whose product must act before gonococcal and meningococcal LOS can be elongated. Using a PCR amplification strategy, in combination with **DNA** sequencing, we demonstrated that N. subflava 44 possessed lgtA, lgtB, and lgtE genes. The predicted amino acid sequence encoded by each of these genes suggested that they encoded functional **proteins**; however, structural analysis of LOS isolated from this strain indicated that the bulk of its LOS was not modified by these gene products. This suggests the existence of an additional regulatory mechanism that is responsible for the limited expression of these genes in this strain.

L13 ANSWER 2 OF 8 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000261945 MEDLINE
DOCUMENT NUMBER: 20261945
TITLE: Sequence variation in the porA gene of a clone of Neisseria meningitidis during epidemic spread.
AUTHOR: Jelfs J; Munro R; Wedege E; Caugant D A
CORPORATE SOURCE: WHO Collaborating Centre for Reference and Research on Meningococci, Department of Bacteriology, National Institute of Public Health, N-0403 Oslo, Norway.
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2000 May) 7 (3) 390-5.
Journal code: CB7. ISSN: 1071-412X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY WEEK: 20000901
AB The ET-15 clone within the electrophoretic type (ET)-37 complex of **Neisseria** meningitidis was first detected in Canada in 1986 and has since been associated with outbreaks of meningococcal disease in many parts of the world. While the majority of the strains of the ET-37 complex are serosubtype P1.5,2, serosubtype determination of ET-15 strains may often be incomplete, with either only one or none of the two variable regions (VRs) of the serosubtype PorA outer membrane **protein** reacting with monoclonal antibodies. **DNA** sequence analysis of the porA gene from ET-15 strains with one or both unidentified serosubtype

determinants was undertaken to identify the genetic basis of the lack of reaction with the monoclonal antibodies. Fourteen different porA alleles were identified among 38 ET-15 strains from various geographic origins. The sequences corresponding to subtypes P1.5a,10d, P1.5,2, P1.5,10d, P1.5a,10k, and P1.5a,10a were identified in 18, 11, 2, 2, and 1 isolate, respectively. Of the remaining four strains, which all were nonserosubtypeable, two had a stop codon within the VR1 and the VR2, respectively, while in the other two the porA gene was interrupted by the insertion element, IS1301. Of the strains with P1.5,2 sequence, one had a stop codon between the VR1 and VR2, one had a four-amino-acid deletion outside the VR2, and another showed no expression of PorA on **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**. Our results reveal that numerous genetic events have occurred in the porA gene of the ET-15 clone in the short time of its epidemic spread. The magnitude of microevolutionary mechanisms available in meningococci and the remarkable genetic flexibility of these bacteria need to be considered in relation to PorA vaccine development.

L13 ANSWER 3 OF 8 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97149287 MEDLINE
DOCUMENT NUMBER: 97149287
TITLE: Cloning, sequencing and expression of an Eikenella corrodens gene encoding a component protein of the lectin-like adhesin complex.
AUTHOR: Yumoto H; Azakami H; Nakae H; Matsuo T; Ebisu S
CORPORATE SOURCE: Department of Conservative Dentistry, Tokushima University School of Dentistry, Japan.. yumoto@dent.dent.tokushima-u.ac.jp
SOURCE: GENE, (1996 Dec 12) 183 (1-2) 115-21. Journal code: FOP. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D70829
ENTRY MONTH: 199704
ENTRY WEEK: 19970403

AB A lectin-like substance (LS), that was isolated from Eikenella corrodens (Ec) 1073, migrated as **proteins** of about 300 and 45 kDa upon **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis** under reducing conditions. In this study, we cloned the gene encoding the 45-kDa **protein** and predicted its structure and function. Based on the N-terminal 23-amino acid (aa) sequence of this **protein**, we cloned the region for its N-terminus. We cloned the entire gene by means of gene walking using polymerase chain reaction and Southern hybridization. The **nucleotide** sequences of cloned

fragments revealed an open reading frame encoding a **polypeptide** of 330 aa (M(r), 35748). This ORF displayed high homology to those of porins of **Neisseria** species. Using the T7-expression system, the 45-kDa **protein** was produced in E. coli. Our results suggested that the 45-kDa **protein** of Ec 1073 is a component of the EcLS complex, and that it is the major outer membrane **protein**.

L13 ANSWER 4 OF 8 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95247686 MEDLINE
DOCUMENT NUMBER: 95247686
TITLE: Interaction of the **Neisseria** gonorrhoeae PilA **protein** with the pilE promoter involves multiple sites on the **DNA**.
AUTHOR: Arvidson C G; So M
CORPORATE SOURCE: Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland 97201-3098, USA..
CONTRACT NUMBER: AI20845 (NIAID)
AI07354 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 May) 177 (9) 2497-504. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508

AB PilA is the putative **DNA**-binding component of a two-component system that regulates transcription of the pilin expression locus (pilE) of **Neisseria** gonorrhoeae. Here we report the purification of the PilA **protein** and characterization of its **DNA**-binding activity. PilA was overproduced in Escherichia coli with an isopropyl-beta-D-thiogalactopyranoside (IPTG)-inducible expression vector. Cell extracts were prepared by sonication and fractionated by anion-exchange chromotography, followed by dye affinity chromatography with Cibacron Blue. **Proteins** were eluted by using a gradient of KCl, and PilA-containing fractions were identified by immunoblot analysis with a polyclonal anti-PilA antiserum. Purified PilA was judged to be > 90% pure, as determined by Coomassie blue staining and **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**. PilA purified in this manner was used to develop a gel retardation assay with a 301-bp fragment containing the pilE promoter (PpilE) and upstream sequences as a probe. A fragment of similar size containing the E. coli aroH promoter was used as a negative control. Competition experiments using a 100- to 1,000-fold excess of unlabelled **DNA** fragments confirmed the specificity of PilA binding to the pilE promoter. To localize the PilA binding site

within the 301-bp PpilE fragment, stepwise deletions were generated by PCR and the fragments were examined in the gel shift assay. The results of these experiments show that there are two regions upstream of PpilE that are required for binding by PilA.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 5 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 94200567 MEDLINE
DOCUMENT NUMBER: 94200567
TITLE: Genetic diversity of the iron-binding **protein** (Fbp) gene of the pathogenic and commensal **Neisseria**.
AUTHOR: Genco C A; Berish S A; Chen C Y; Morse S; Trees D L
CORPORATE SOURCE: Department of Microbiology and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310..
CONTRACT NUMBER: AI30797 (NIAID)
SOURCE: FEMS MICROBIOLOGY LETTERS, (1994 Feb 15) 116 (2) 123-9.
Journal code: FML. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
AB The pathogenic **Neisseria** and most commensal **Neisseria** species produce an iron-binding **protein** (Fbp) when grown under iron-limited conditions. In the current study, we confirmed the presence of Fbp, as well as **DNA** sequences homologous to the gonococcal fbp, in strains of N. gonorrhoeae, N. meningitidis, N. cinerea, N. lactamica, N. subflava, N. kochii and N. polysaccharea. The fbp genes from these strains were amplified by the polymerase chain reaction, digested with StuI or RsaI, and the restriction patterns examined. The patterns for the gonococcal and meningococcal fbp were virtually identical; however, variations were observed in the fbp sequences of the commensal **Neisseria** species. N. flavescens, N. mucosa, N. sicca, N. ovis and Branhamella catarrhalis, did not produce Fbp as detected by **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis** and reactivity with an Fbp specific monoclonal antibody, nor did they hybridize to an fbp-specific **DNA** probe.

L13 ANSWER 6 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94059745 MEDLINE
DOCUMENT NUMBER: 94059745
TITLE: Neisseria weaveri sp. nov. (formerly CDC group M-5), from dog bite wounds of humans.
AUTHOR: Holmes B; Costas M; On S L; Vandamme P; Falsen E; Kersters K

CORPORATE SOURCE: Epidemiological Identification and Typing Laboratory, Central Public Health Laboratory, London, United Kingdom..
SOURCE: INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, (1993 Oct) 43 (4) 687-93.
Journal code: AWO. ISSN: 0020-7713.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
AB The taxonomic relationships of strains belonging to Centers for Disease Control group M-5 were examined. Previous studies of rRNA cistron similarities placed this organism on the Neisseriaceae rRNA branch of rRNA superfamily III; the closest neighbors included the genus **Neisseria** and groups EF-4a and EF-4b. The group M-5 strains were characterized by a range of phenotypic tests, and their G + C contents and **DNA-DNA** relatedness levels were determined. In addition, a numerical taxonomic analysis of the whole-cell **protein** patterns (obtained by **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**) of group M-5 and related taxa was performed. The strains studied included 45 group M-5 strains, the type strains of six **Neisseria** species or subspecies, three group EF-4a reference strains, and three group EF-4b reference strains plus the type strain of the phenotypically similar organism Oligella urethralis. Our results showed that the group M-5 strains were members of a homogeneous taxon distinct from phylogenetically closely related taxa. The genomic divergence as revealed by levels of rRNA cistron similarity and phenotypic characteristics indicate that group M-5 can be considered a new species of the genus **Neisseria**. We therefore propose the new species **Neisseria** weaveri, with NCTC 12742 (= CCUG 4007 = ISL775/91 = LMG 5135) as the type strain. N. weaveri strains are strictly aerobic, gram-negative, nonmotile, rod-shaped organisms which are catalase and oxidase positive, nonsaccharolytic, and able to grow on MacConkey agar and do not reduce nitrate but generally reduce nitrite.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 7 OF 8 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 93084746 MEDLINE
DOCUMENT NUMBER: 93084746
TITLE: Identification of meningococcal serosubtypes by polymerase chain reaction.
AUTHOR: Maiden M C; Bygraves J A; McCarvil J; Feavers I M
CORPORATE SOURCE: National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom..
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1992 Nov) 30 (11) 2835-41.

Journal code: HSH. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303

AB The polymerase chain reaction was used as the basis of a novel typing method for **Neisseria** meningitidis. Southern hybridization experiments demonstrated that it was possible to identify genes encoding different serological variants of the meningococcal class 1 outer membrane **protein** by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14 defined variable regions was prepared in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane **protein** genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one experiment. This technique was evaluated with a set of serological reference strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis** or serological analysis. An additional advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the **DNA** amplified from the original sample could be used to determine the **nucleotide** sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clinical samples even when culture is not possible because of antibiotic treatment of an acute case.

L13 ANSWER 8 OF 8 MEDLINE
ACCESSION NUMBER: 86149857 MEDLINE
DOCUMENT NUMBER: 86149857
TITLE: **Polypeptides** encoded by cryptic plasmids from **Neisseria** gonorrhoeae.
AUTHOR: Aalen R B; Gundersen W B
SOURCE: PLASMID, (1985 Nov) 14 (3) 209-16.
Journal code: P8P. ISSN: 0147-619X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198606

AB Almost all clinical isolates of **Neisseria** gonorrhoeae

harbor a small, phenotypically cryptic plasmid of approximately 4.1 kb. In this study several **polypeptides** encoded by two variants of such plasmids, one (pSB01C) having a deletion of approximately 50 bp as compared to the other (p31788C), have been identified, and the position of the genes for two of the **proteins** determined. The cryptic plasmids were cloned into the HindIII site of the vectors pBR322 and pACYC184. The resulting recombinant plasmids were transformed into the Escherichia coli minicell producing strain DS410 (minA, minB) and the plasmid-encoded **proteins** analyzed by **sodium dodecyl** sulfate-**polyacrylamide** gel **electrophoresis**. The pSB01C derivatives express two distinct **proteins** of 22 and 16 kDa and p31788C two other **proteins** of 24 and 18.5 kDa. Additionally, both plasmids express common **proteins** of 32.5, 9, and 7.5 kDa. The genes coding for the 24- and the 7.5 kDa **proteins** have been mapped by restriction enzyme analysis of Tn5 insertions suppressing the expression. The additional 50 bp in p31788C are localized to the coding region of the 24-kDa **protein**, and the 22-kDa **protein** of pSB01C is possibly a shortened form of the former due to the lacking 50 bp.

FILE 'HOME' ENTERED AT 10:03:57 ON 22 MAR 2001

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     FILE 'CAPLUS' ENTERED AT 10:20:57 ON 22 MAR 2001
L4          1688 SEA FILE=CAPLUS ABB=ON  PLU=ON  NEISSERIA(S)(POLYPEPTIDE
                 OR PEPTIDE OR PROTEIN OR POLYPROTEIN)
L5           609 SEA FILE=CAPLUS ABB=ON  PLU=ON  L4 AND (DNA OR DEOXYRIBON
                 CULEIC OR DEOXY RIBONUCLEIC OR NUCLEOTIDE)
L14        51368 SEA FILE=CAPLUS ABB=ON  PLU=ON  (SDS OR (NA OR SODIUM)(W)
                 DODECYL)(1W)(PAGE OR (POLYACRYL? OR POLY ACRYL?)(3W)ELECT
                 ROPHOR?)
L15           24 SEA FILE=CAPLUS ABB=ON  PLU=ON  L5 AND L14


L16            0 S L15 NOT (L6 OR L9)

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT,
     TOXLINE, PHIC, PHIN' ENTERED AT 10:31:15 ON 22 MAR 2001
L17           72 S L15
L18            0 S L17 NOT (L7 OR L12)

=> fil hom

FILE 'HOME' ENTERED AT 10:33:35 ON 22 MAR 2001
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